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Introduction to  
**MICROTECHNIQUE**  
of  
**INORGANIC ANALYSIS**



# Introduction to the MICROTECHNIQUE of INORGANIC ANALYSIS

BY

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## PREFACE

This volume replaces the *Introduction to the Microtechnique of Inorganic Qualitative Analysis* by A. A. Benedetti-Pichler and W. F. Spikes.<sup>1</sup> Like its precursor it is intended as an introduction to the microtechnique of chemical experimentation. The presentation of chemical procedures and analytical schemes has been reduced to a minimum because of the conviction that approved methods may be simply and successfully transposed to any scale, no matter how small, provided that a suitable technique of working and observing is available. In recent years chemical reactions have been performed in drops so small that they could no longer be seen with the unaided eye, but no conclusive evidence has been discovered which would have indicated a radical shift in the chemical equilibria. When working with relatively large quantities (0.00001 to 1 mg), strict adherence to the hypothesis of the independence of chemical equilibria from absolute mass or volume will prove useful in spite of exceptions which may be discovered in the future.

Centigram, milligram, and microgram techniques of qualitative analysis have been included in the text so as to provide for a desirable variety. Spot tests have been described, which may take the place of slide tests if microscopes are either not available or not available in sufficient numbers. The decision to add a section on quantitative microanalysis was made when it was found that precise analytical balances may take the place of microchemical balances. Microburets of a type which can be made at small expense offer another possibility for widening the scope of quantitative work.

All directions are given in great detail. This will enable instructors to supervise the work of a large number of students. Experienced chemists who wish to acquire the technique of microanalysis may use the book as their guide. They should resist the temptation to limit their study to a few experiments which seem to be an immediate preparation for the contemplated practical use. There is no substitute for personal experience with a wide variety of methods. The rudiments of qualitative technique should be acquired, even if one is interested in quantitative applications only. For the use of those who have

<sup>1</sup> Microchemical Service, Douglaston, N. Y., 1935.

no training in microscopy a brief description of the microscope and instructions for its care and use have been included in the text. Information concerning apparatus, test substances, unknowns, and reagents is compiled in the Appendix. For other subject matter the reader is invited to make extensive use of the Index, which has been prepared with considerable care.

The experiments described have been tested by the author or his coworkers. The general presentation of the subject has been greatly influenced by the work of Dr. Julian R. Rachele on the limits of identification of simple chemical tests. Miss Anne G. Loscalzo, Mr. Richard N. Boos, and Mr. Michael Cefola have perfected the methods of qualitative separation. Miss Anna-Greta Hybbinette, fellow of the Swedish-American Foundation, Drs. Alberto J. Llacer and Juan A. Sozzi, fellows of the Asociación Argentina para el Progreso de las Ciencias, Mr. R. A. Paulson, and Mr. S. Siggia are to be credited for the development and testing of the quantitative procedures. Most of this experimental work was carried out at the Washington Square College of New York University, and it is with pleasure that I express my gratitude to Professor William C. MacTavish, Administrative Chairman of the Chemistry Department, for the generous manner in which apparatus and facilities were placed at my disposal. Furthermore I am indebted to the administration of Queens College for providing facilities for the experimental work with microburets carried out by Miss A.-G. Hybbinette, Drs. Llacer and Sozzi, and Mr. Siggia.

A. A. BENEDETTI-PICHLER

*March, 1942*

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## INTRODUCTION

Microchemistry may be defined as the systematic knowledge of the techniques suited for the performance of chemical experiments on an essentially smaller scale than is usual in laboratory practice. General microchemistry deals with the development, testing, correlation, and systematization of the methods which are needed for the handling and for the observation or determination of properties on small amounts of matter. Microanalysis is a branch of applied microchemistry, i.e., it is the application of the microtechnique of chemical experimentation to the solution of analytical problems.

The reason for the predominant position of experimental technique in microchemistry is quite obvious. It is generally agreed that, aside from temperature and pressure, homogeneous chemical equilibria are mainly determined by the active molar concentrations of the reacting substances. The size, i.e., volume or mass, of the system is of no importance as long as the number of reacting molecules statistically assures the establishment of average conditions. In general one may expect that, as far as the major constituents are concerned, a "quantitative" reproduction of the usual macro conditions of equilibrium can be obtained with one million reacting molecules, whereas "qualitative" duplication may be satisfactory with one hundred molecules. These estimates correspond to  $10^{-16}$  and  $10^{-20}$  gram of reacting matter, if an average molecular weight of 100 is assumed. Identical conditions of temperature, pressure, and active concentration being provided, chemical reactions will always produce the same result as long as not less material is taken than indicated by the above limits.<sup>1, 2</sup> Thus, the reactions of microchemistry, in which usually more than trillions of molecules take part, will in no way differ from those observed with large quantities, and the distinctive features of microchemistry are restricted to the experimental technique required.<sup>3</sup> It is implied that every chemical process can be carried out on a small

<sup>1</sup> F. Emich, *Lehrbuch der Mikrochemie*, Munich, J. F. Bergmann, 1926, p. 2, line 1.

<sup>2</sup> A. A. Benedetti-Pichler and J. R. Rachele, *Ind. Eng. Chem., Anal. Ed.*, 12, 233 (1940).

<sup>3</sup> P. Harting, *Das Mikroskop*, etc., Braunschweig, Fr. Vieweg u. Sohn, 1859, p. 437.

scale if the proper methods of manipulation and observation can be developed.

Small is a relative term. One-tenth of a gram may be a very small quantity for an organic synthesis, but it is the usual amount for most analytical work. This raises the question of the borderline between microchemical and macrochemical work. It should be realized that a sharp distinction has no practical importance, since the prefixes macro, semi-micro, micro, and ultramicro possess neither derogatory nor complimentary implications. There is no doubt, however, that the modifier "micro" should be used if the amount of matter is sufficiently small to render the customary methods of handling or observation unreliable or inefficient.

The experimental technique used with milligram quantities may require modification for application to ten times smaller or larger amounts, and it is, as a rule, not applicable to quantities a thousand times smaller or larger. Accordingly, microchemistry must offer a variety of techniques for each operation, and it is logical to classify them on the basis of the amount of material involved. The suggestion of Emich to forego romantic appeal in favor of clarity is adopted in this book, and the methods are classified as centigram, milligram, and gamma procedures when the masses for which they are best suited are approximately of the order of a centigram, a milligram, or a gamma. It must be added that the criterion for classification is given by the quantity of material at the *start* of the procedure, that is, by the mass of the *sample* for analytical procedures and not by the mass of constituent contained in the sample. Of course, the isolation of traces usually requires work on a gradually decreasing scale. It is obvious that gamma procedures cannot be used at the outset, when  $3\gamma$  of magnesium are to be isolated from 10 grams of aluminum metal. The elimination of the bulk of aluminum will require gram procedures (macro methods); the separation of the magnesium from other trace constituents may necessitate the use of milligram procedures, and the exact determination of the isolated magnesium definitely calls for the application of a gamma technique.

If known, the most favorable conditions of concentration, temperature, and pressure should be used in microchemical work. As a rule, it will be advisable to adhere as strictly as possible to the prescriptions of generally used standard procedures, since all chemical knowledge concerning their reliability, advantages, and shortcomings may then be directly applied to the micro work.

In the transposing of procedures from one scale to another, special attention must be paid to the maintenance of concentrations. This is

simple enough in analytical work, since it is only necessary to multiply all masses and volumes of reagents as well as the capacities of apparatus (beakers, flasks, burets, etc.) by the ratio

$$\frac{\text{Mass of sample on new scale}}{\text{Mass of sample on former scale}}$$

and to leave the concentrations unchanged. Frequently, it will happen that the calculated volumes become smaller than "compatible with efficient manipulation," and the experimenter decides to work with "somewhat" larger volumes. Obviously, such a decision means a deviation from the standard procedure and may cause serious errors. At the same time, the development of a proper microtechnique has been evaded.

Brief consideration shows that the purity specifications of the reagents also must be retained when a procedure is properly transposed from one scale to another. In the isolation of traces, extraordinary requirements of purity must be met by those reagents which are used in large quantities during the initial stages of the process.

The difficulties encountered in transferring to a smaller scale may be attributed to various causes. At times, the macro procedure has minor shortcomings of such kind that their effect is not reduced in the same proportion as the size of the sample; at once, unnoticeable irregularities of the macro scale introduce grave uncertainties into the micro procedure. The changes of weight on heating porcelain, quartz, and platinum apparatus to high temperatures belong in this category. The lack of suitable materials for the construction of tools may hinder the application of a technique which has been recognized as best suited to the problem. To give one example, a perfectly transparent material which could withstand the combined action of hydrofluoric acid, sulfuric acid, and heat would be of great service to qualitative microanalysis. Another factor deserving general attention is the increase of relative surface which accompanies every shift to a smaller scale. If **relative surface** is defined as the ratio surface over volume of the solution under investigation, it must increase approximately ten times when changing to a scale a thousand times smaller.<sup>4</sup> The possible consequences are obvious. The relatively greater area of contact with the walls of the apparatus may increase the extent of chemical attack and cause excessive contamination of the sample. The exchange of gases and vapors through the relatively enlarged liquid-gas interface may be of disadvantage. Thus, the suitability of any microchemical apparatus may be judged by whether or not its

<sup>4</sup> A. A. Benedetti-Pichler, *Ind. Eng. Chem., Anal. Ed.*, **9**, 483 (1937).

surface and volume (and weight, if used in gravimetric determinations) have been reduced in approximately the same proportion as the mass of the sample. Of course, that part of the surface which does not come in contact with the investigated material and the reagents does not enter the estimation, provided that the apparatus need not be weighed. It is understood that in working with extremely small quantities of material the effect of surface forces must be given continuous consideration in the development of the manipulative technique.

Although François Vincent Raspail was probably the first to conceive and apply the principles of microanalysis,<sup>5</sup> the systematic development of microchemical experimentation has been the work of F. Emich, who based his investigations upon the general premises which have been discussed in the preceding paragraphs. The forceful logic and the universal applicability of Emich's reasoning and his personality as a scientist and teacher have established an international school of microchemistry. Thus, it may be easily understood why this book had to be built, with a few minor exceptions, entirely upon the work of Emich and his students of first and second generation.

<sup>5</sup> E. M. Chamot and C. W. Mason, *J. Chem. Education*, 5, 258 (1928). Raspail studied the formation of starch in the organs of plants. In 1825 he proceeded to isolate the objects of investigation by microdissection, and to perform chemical tests under the microscope.

***PART I***



## APPARATUS FOR GENERAL USE

### LABORATORY AND WORK BENCH

Training in the microtechnique of chemical analysis does not require a special laboratory. The efficient performance of microchemical routine work, however, or continuous work on microchemical problems, necessitates properly equipped rooms which can be kept meticulously clean. The most outstanding general requirements listed in the order of decreasing importance are absence of dust and fumes, constant temperature, absence of troublesome vibrations, and reasonably constant humidity of the atmosphere. For a great deal of the work, the experimenter should be comfortably seated and able to steady his hands by resting the arms on the table top. Much information on the design of microchemical laboratories has been furnished by H. K. Alber and J. Harand,<sup>1</sup> B. L. Clarke and H. W. Hermance,<sup>2</sup> J. B. Peterson and E. W. Schoeffel,<sup>3</sup> and E. F. Shelberg.<sup>4</sup>

### GAS BURNERS

Bunsen burners are required in many experiments. First choice should be given a Bunsen burner with a slender pilot flame of 5- to 10-mm height, which serves as microflame. If burners with suitable pilot flames are not available, a second Bunsen burner from which the barrel has been removed may be used as microburner; the gas is lighted at the jet, and the height of the flame may be regulated by means of a screw clamp on the rubber tubing if the burner is not provided with a valve for regulation.

Very handy microburners (Fig. 1) may be made from glass tubing by drawing out one end of the tubing to a capillary of the desired bore, bending the capillary, and sealing two glass prongs to the wide part of the tube. For the regulation of the microflame a screw clamp and a loose plug of cotton within the rubber tubing will serve. The size of the flame is determined by the diameter of the opening of the

<sup>1</sup> H. K. Alber and J. Harand, *Ind. Eng. Chem., Anal. Ed.*, **10**, 403 (1938).

<sup>2</sup> B. L. Clarke and H. W. Hermance, *ibid.*, **7**, 218 (1935).

<sup>3</sup> J. B. Peterson and E. W. Schoeffel, *ibid.*, **10**, 172 (1938).

<sup>4</sup> E. F. Shelberg, *ibid.*, **10**, 704 (1938).

capillary. A very small flame is obtained as follows:<sup>5</sup> The micro-flame is lighted and the illuminating gas is turned on full so as to obtain the maximum height of the flame. Then the opening of the capillary is heated by playing the edge of a roaring Bunsen flame on it until the height of the microflame has been reduced to 5 mm. Regu-

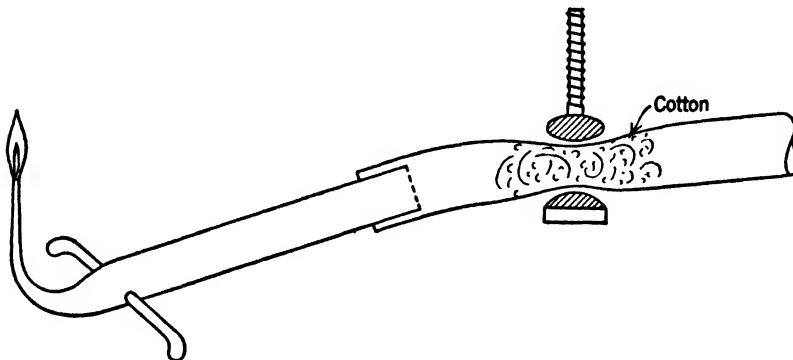


FIG. 1. Microburner, natural size.

lation of the screw clamp will now permit obtaining a microflame the size of a pinhead.

#### GLASS KNIVES

For the proper use and maintenance of glass knives one should follow the directions of Professor Carl Benedicks of the Metallografiska institutet in Stockholm.<sup>6</sup> As a rule, however, it will be sufficient to use a triangular file for the cutting of larger tubing. The sharp edges of pieces of broken porcelain or glazed earthenware are very efficient for the cutting of capillaries and fine glass threads.

#### EXPERIMENT 1

##### Preparation of Wash Bottles

Required for 1 wash bottle:

- 1 Erlenmeyer flask, Pyrex, 125-ml.
- 1 two-hole rubber stopper No. 4.
- 35 cm tubing of 6-mm outside diameter.

The center of the glass tubing is heated in the roaring flame of a Bunsen burner and drawn out to a capillary of approximately 10- to

<sup>5</sup> Ruth B. Howland and M. Belkin, *Manual of Microurgy*, New York, New York University Press Book Store, 1931.

<sup>6</sup> H. K. Alber, *Mikrochemie*, 18, 94 (1935).

20-cm length and of not less than 1-mm bore. The capillary is cut so that a 12-cm length of it remains with one piece of tubing to form the nozzle of the wash bottle. The tubes are then bent and cut to proper form and the dimensions indicated in Fig. 2, and all the cut surfaces are carefully firepolished. It is understood that the opening of the capillary tip will collapse slightly when heated; to prevent complete sealing of the opening, the end of the capillary is heated for a second at a time in the edge of the lower part of the Bunsen flame.

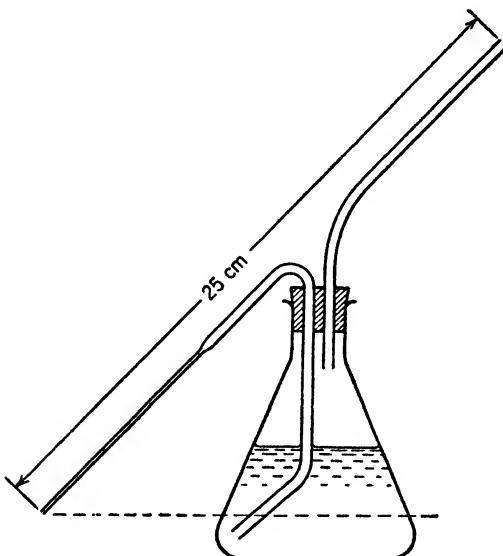


FIG. 2. Wash bottle.

The stopper must consist of live rubber which does not give off particles. The protective coating is removed with a cloth soaked in carbon tetrachloride. The holes are treated with a trace of glycerin before the glass tubes are inserted. A wad of cotton is wound around the knurled end of a probing wire and dipped into glycerin. Most of the glycerin is removed by pressing the wad between the fingers, and the wad is pushed through the holes of the stopper. The holes of the stopper are then wiped by pushing a wad of clean cotton through. Now the glass tubes may be inserted without the application of much force.

The illustration shows the opening of the nozzle below the level of the water in the flask. When the tip is once filled with water, it stays that way, since the surface tension holds the liquid in the capillary. The water level in the flask is adjusted so that, without

blowing, a slight inclining of the wash bottle causes delivery of liquid from the tip.

## EXPERIMENT 2

### Preparation of Capillaries

Soft glass tubing of 8-mm bore, or, if a blast lamp is available,

Soft glass tubing of 10- to 15-mm bore.

Feathers.

Soap solution; paper towels.

Capillaries and threads made of a glass of high elasticity give satisfaction. Various kinds of soft glass differ in this respect, and it seems advisable to perform a test before acquiring a supply of tubing for microchemical use. Comparison of the behavior of capillaries or threads of approximately 0.2-mm outer diameter made from the tubing in question and from tubing which has given satisfactory service will suffice. Pyrex glass is very brittle, and its use for the preparation of fine capillaries and threads should be avoided. Clear fused quartz is recommended for its resistance to chemical attack, its tensile strength, and its remarkable elasticity.

Before being drawn into capillaries, glass tubing must be carefully cleaned. The tubing is cut into lengths of 15 to 20 cm, and then cleaned inside and outside with soap solution and a feather long enough to reach the whole inside of the piece of tubing when inserted once at one end and once at the other. The pieces of tubing are rinsed with tap water and then with distilled water. They are finally collected in bundles which are wrapped in paper towels and placed in an upright position for draining and drying. The tubes are kept wrapped in paper until all of them have been used up.

The roaring flame of a Bunsen burner suffices for the drawing of capillaries from tubing of 8-mm or smaller bore. A piece of tubing is grasped at both ends and heated just above the blue cone of the flame while being slowly but continuously rotated around its axis. When the glass has become soft, the tubing is removed from the flame and leisurely drawn out so as to give a capillary of approximately 0.5- to 1-mm bore. The rate of drawing determines the bore and the length of the capillary obtained. It is necessary to watch the thread of glass closely while drawing and to regulate the rate of drawing according to requirements. The pull must not be released before the glass has hardened; otherwise bent capillaries will result.

The capillary is cut as shown in Fig. 3A, so that a piece *a* is left with the tubing of large bore; *a* is used for a handle when drawing the

next piece of capillary from part *b* of the tubing. This procedure gives lengths of capillaries separated by small bulbs, Fig. 3*B*. The capillaries are cut in such a way that 6-cm lengths of capillary remain on either side of the bulbs. The small pipets thus obtained are useful in transferring relatively large volumes of liquids and should be saved. The supply of capillary tubing should be cut into lengths of not less than 20 to 30 cm. The capillaries are sealed at both ends and kept in a box or tube until needed. Capillaries of 0.5- to 1-mm inner diameter are most useful; capillaries of smaller bore should be saved too, for they can be used occasionally.

Left-over pieces of tubing of the form shown in Fig. 3*A* are sealed together at the wide ends, forming short pieces of tubing with capi-

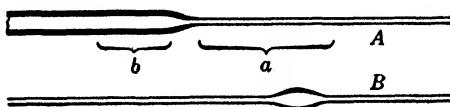


FIG. 3. Drawing of capillaries.

lary handles at both sides. These handles make it possible to work the left-over pieces completely into capillaries.

Great lengths of capillary can be obtained in a short time from wide glass tubing of 10- to 15-mm bore with the use of the large blue flame of a blast lamp. One person heats the tube in the flame, and, when the glass has softened and has been taken out of the flame, a second person grasps one end of tube and walks quickly away with it while the other end is held steadily by the first person. Three to ten yards of capillary of quite uniform bore can be obtained at one time. The speed of drawing depends upon the desired bore, upon the inner diameter and the thickness of wall of the original tubing, upon the temperature of the glass, and upon the width of the flame, which determines the length of tubing that acquires the necessary plasticity.

#### CENTRIFUGE CONES AND MICROCONES

Centrifuge tubes of any size should be made of Pyrex glass, which is well able to withstand mechanical stress. Tubes of two sizes are used in the work considered here. The centrifuge cones shown in Fig. 4*A* have a capacity of 2.5 to 3 ml and are used in centigram procedures; the microcones, Fig. 4*B*, have a capacity of approximately 0.7 ml and serve for the qualitative analysis of milligram samples. The inside dimensions are indicated in the figures. The straight-tip cones,

Fig. 4*C*, serve for the estimation of the volume of precipitates in milligram procedures and for the performance of extractions. The capillary part must be of uniform bore. The bore is specified depending upon the requirements, usually within the limits from 1 to 3 mm.

Stirring rods for centrifuge cones are obtained by heating one end of a capillary of approximately 9-cm length and 1-mm bore in the edge of a roaring Bunsen flame. First the capillary is rotated until a

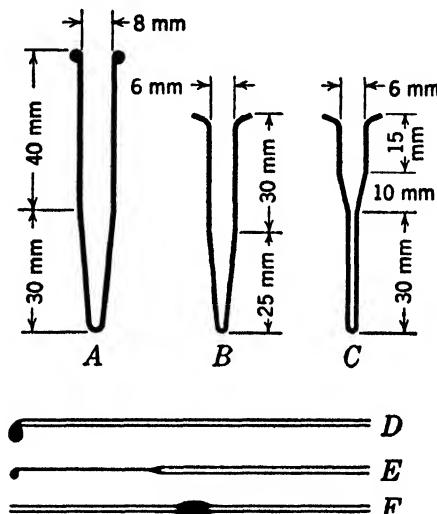


FIG. 4. *A*, centrifuge cone; *B*, microcone; *C*, straight-tip cone.

bead of glass has formed; then the capillary is held quiet and horizontal, the end touching the flame, until the bead has dropped in the position shown in Fig. 4*D*. Stirring rods for microcones, Fig. 4*E*, are freshly prepared when needed. The center of a short piece of capillary is fused into a bead by heating in a microflame or in the edge of a Bunsen flame while the capillary is slowly rotated around its axis, Fig. 4*F*. The capillary is removed from the flame and the bead is drawn out to a glass thread a few tenths of a millimeter in diameter. The thread is broken at a distance of 30 mm from the capillary handle, and the end of the thread is fused into a tiny glass bead which is finally allowed to droop to one side, Fig. 4*E*. Stirring rods for straight-tip cones are held in a perpendicular position over a small microflame when the bead is fused; only very small beads are permissible in this instance, and their centers must lie in the axes of the stirring rods.

**CENTRIFUGES**

Small hand-driven centrifuges are most efficient for work in microcones of 0.7-ml capacity. They can be rapidly started and stopped, and a few turns of the crank often serve the purpose. Sturdy electric centrifuges are preferable when most of the work is performed with the somewhat heavy centrifuge cones of 3-ml capacity. The high speed of electric centrifuges is at times required with milligram procedures, and it is of definite advantage when working in capillary cones of  $1-\lambda$  capacity.

Every centrifuge should be provided with a shield having an easily removable and replaceable cover. Provision for simple and rigid attachment to any kind of table top is another essential feature. Exchangeable heads for different types of centrifuge tubes are very useful. A microhead suggested by Emich and shown in Fig. 5B increases the efficiency of a centrifuge for micro work. The long slots in the metal bar of the head facilitate the centrifuging of long capillaries, and placement of the metal shells at the ends of the relatively long arms increases the centrifugal force which is directly proportional to the distance between the axis of rotation and the point of the cone. The makeshift arrangement of Fig. 5A, which uses the aluminum shell of a clinical hand centrifuge, is decidedly less satisfactory as may be seen by a comparison of the radii  $r$  in Figs. 5A and 5B.

Centrifuge cones of 3-ml capacity and larger centrifuge tubes are always placed in closed metal shells for centrifuging, and, as a rule, the glass cones rest on their points. A rubber cushion or a plug of cotton placed on the bottom of the metal shell will prevent breakage of the glass tube. Should the tube collapse during centrifuging, the metal shell must be immediately and thoroughly cleaned to prevent corrosion and to remove all glass splinters, which might otherwise cause the destruction of some more cones. Whenever centrifuge tubes of soft glass or of very thin glass are employed, it is best to fill the space between metal shell and glass cone with water. In this way the pressure is evenly distributed over the walls of the more or less floating centrifuge cone.

The metal shells for microcones of 0.7-ml capacity may be either open or closed at the bottom as shown in Figs. 5B and 5C. The closed shells are used to float the microcones in water during swirling, so as definitely to exclude the possibility of breakage. Microcones of Pyrex

glass break very rarely, however, and this danger may be still further decreased by placing a rubber band, cut from tubing of suitable bore, below the rim of the cone as indicated in Fig. 5C. The open metal shell of Fig. 5C is more generally useful, since the length of glass apparatus to be centrifuged is mainly limited by the radius of the *shield* of the centrifuge.

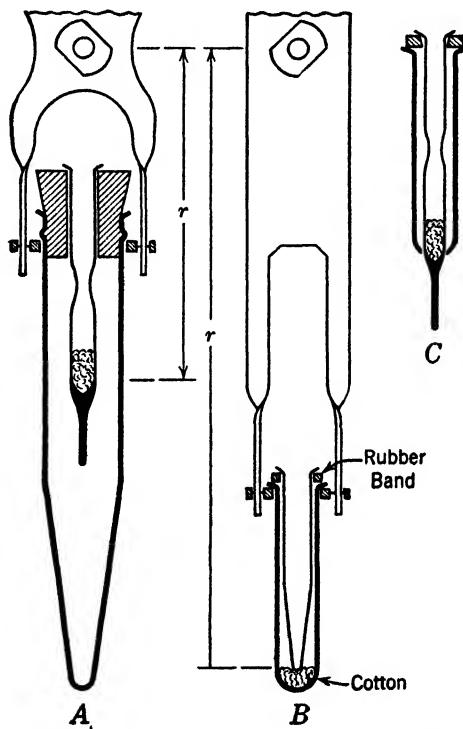


FIG. 5. Micro attachments for centrifuges.

It is understood that the masses at the ends of the centrifuge head must be balanced before starting the centrifuge. With microcones it is sufficient to insert glass cones of equal size on opposite sides of the head. When working with centrifuge cones of 3-ml capacity or even larger tubes, the equalizing of masses must be performed with the use of a trip balance. The metal shells containing the glass tubes are placed on the pans of the balance, and water is added to the tube serving as tare, until equilibrium is established.

Centrifuges must be started and stopped gradually. The cover is to be placed on the shield before starting, and it must not be removed before the centrifuge has come to a stop.

$$\text{Centrifugal force} = f = 0.011 \text{ } rmn^2 \text{ dynes}$$

$$\text{Relative centrifugal force} = 1.2 \times 10^{-5} \text{ } rn^2$$

when  $r$  is the radius of the orbit of revolution in centimeters,  $m$  is the mass of the revolving body in grams, and  $n$  is the number of revolutions per minute. The relative centrifugal force<sup>7</sup> is the ratio of centrifugal force to gravitational force acting on the same mass.

### WOODEN BLOCKS

Hardwood blocks of the type shown in Fig. 6 occupy little space and are very convenient for use with various kinds of microtubes.

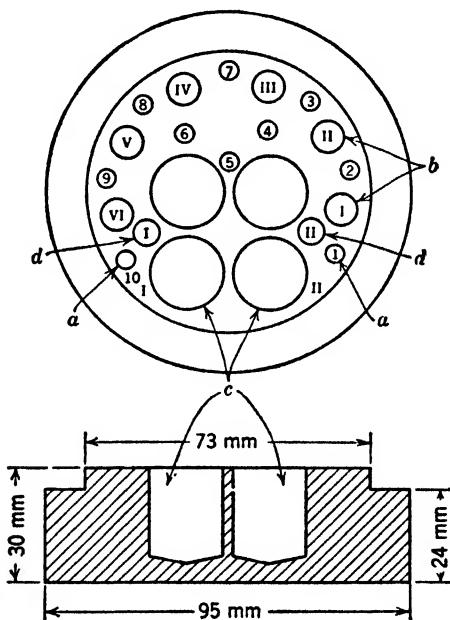


FIG. 6. Block of hardwood. *a*, ten holes for microcones, 5-mm diameter, 20-mm depth; *b*, six holes for centrifuge cones, 8-mm diameter, 25-mm depth; *c*, four holes for microbreakers, 18-mm diameter, 25-mm depth; *d*, two holes for filtersticks, 6-mm diameter, 25-mm depth.

The large holes I and II are intended for microbreakers employed in quantitative work. The two large holes behind them may be used for tare bottles, and the smaller holes I and II are for filtersticks. Six medium-sized holes, I to VI, have the proper dimensions for 3-ml

<sup>7</sup> A nomogram for rapid estimation has been published by H. Schapiro, *Ind. Eng. Chem., Anal. Ed.*, 7, 25 (1935).

centrifuge cones. When working with microcones of 0.7-ml capacity, the smallest holes, 1 to 10, are to be used. Since only one type of tube is used at a time, the apparatus will not be inconveniently crowded. The dimensions of the block have been selected so that an inverted Pyrex beaker, Griffin, low form, of 400-ml capacity, can be used as a bell jar. The beaker has the proper height to accommodate the above-listed types of apparatus, and it rests securely on the ring surface 6 mm below the top of the block so that the block with the tubes and the cover can be safely carried in one hand.

#### HEATING DEVICES

A great number of heating devices are in use. The advantages of electrically heated aluminum blocks cannot be denied, but the simple

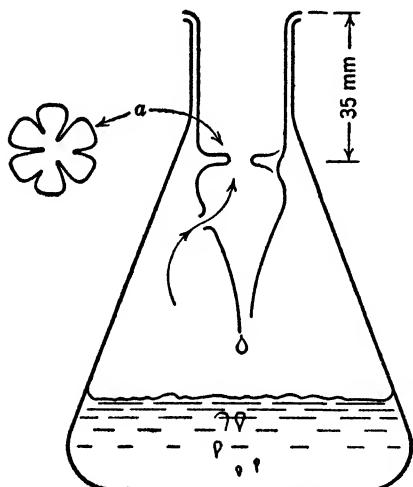


FIG. 7. Steam bath.

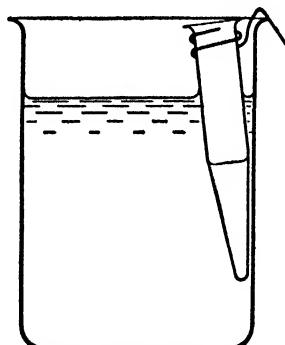


FIG. 8. Water bath.

steam bath shown in Fig. 7 will serve admirably. It consists of a 250-ml Pyrex Erlenmeyer flask, with a narrow mouth, and a rather closely fitting insert which can be easily made of Pyrex glass tubing of 24-mm outer diameter. The cross section of the insert at the level *a* is shown in the side figure. The glass horns pointing toward the center of the insert tube support the cones and microbeakers to be heated. If not too much water is placed in the Erlenmeyer flask, steam can be obtained in a short time. A Bunsen burner or an electric hot plate may be used for heating.

To obtain temperatures below 100° C, a suitable bath liquid in a 150-ml beaker may be used. The centrifuge cone may be attached to the rim of the beaker by means of strong wire of Nichrome or platinum, Fig. 8. An inexpensive short thermometer may be used for stirring the bath liquid.

Metal heating blocks offer the simplest means for the maintenance of controlled temperatures above 100° C. In addition to a well

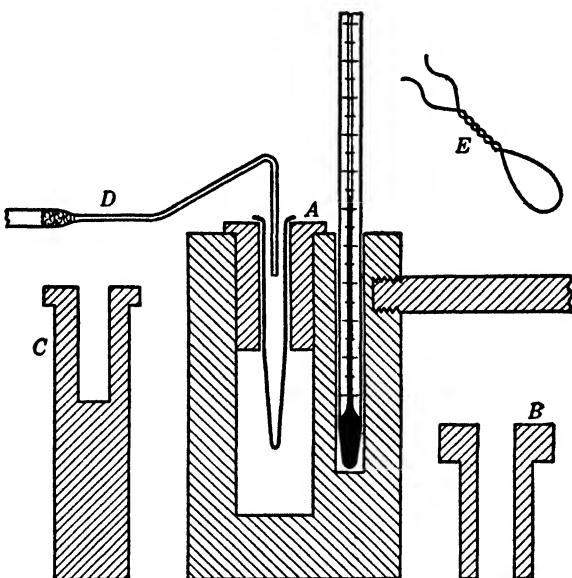


FIG. 9. Heating block. *A*, inset for microcones; *B*, inset for centrifuge cones; *C*, inset for distilling flask, Fig. 44. Approximately one-half natural size.

for the thermometer, they must be provided with holes for the insertion of the various types of apparatus. They are best made of aluminum, which not only is a good conductor but also has a remarkable resistance to the action of corrosive agents. Copper blocks become a nuisance because of the oxide scales which, on heating of the blocks, are frequently ejected by the oxidized surfaces with such force that they are liable to be thrown into the reaction vessels. Electrically heated blocks are more easily kept clean, but heating with a gas flame is perfectly satisfactory for most purposes.

The model of a heating block suggested by Fig. 9 offers the advantage that exchange of the insets *A*, *B*, *C*, etc., permits accommodating centrifuge cones, microcones, distilling apparatus, and microbeakers. The particular form of the insets shown causes the upper portions of the cones to be more rapidly heated than the points. A

fork *E* is easily made of aluminum wire, and it is convenient to use in the removal of cones from the hot block.

For heating under pressure, microcones and centrifuge cones may be sealed with the use of Rachele's pressure cap <sup>8</sup> shown in Fig. 10. The cone rests in a brass ring with rubber lining, to which a metal

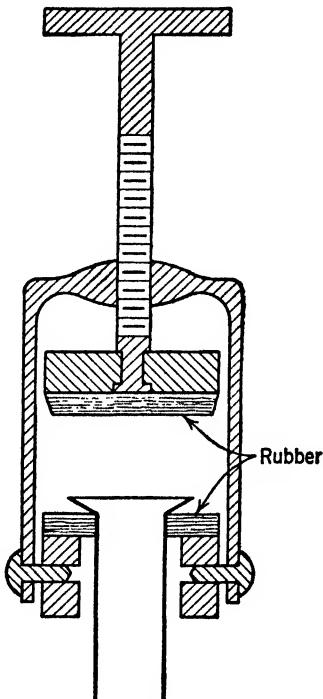


Fig. 10. Rachele's pressure cap applied to microcone.

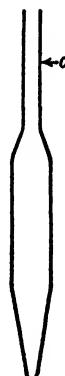


Fig. 11.  
Pressure cone.

stirrup is attached. The rubber-lined pressure plate is tightened on the rim of the cone by means of a screw threaded through the center of the stirrup. As a substitute, the cone may be closed with a rubber stopper which is then secured by means of a ligature made with copper wire of approximately 0.5-mm diameter.

If the use of rubber is not permissible, the solution may be sealed into a pressure cone, Fig. 11, which is given approximately the same dimensions as those of microcones or centrifuge cones. The tube *a* should have a bore of not less than 2 mm to facilitate the introduction of capillary pipets or siphons.

<sup>8</sup> A. A. Benedetti-Pichler and J. R. Rachele, *Ind. Eng. Chem., Anal. Ed.*, **9**, 589 (1937).

**BUZZER FOR SEVERE AGITATION <sup>8</sup>**

An electromagnetic door buzzer, the required transformer, and a switch are mounted on a board and connected to the 110-volt alternating-current line. The interrupter of the buzzer is fastened down so that it cannot make and break. When the buzzer is connected to the 110-volt line through the transformer, the interrupter vibrates with an almost imperceptible motion. If a centrifuge cone, microcone, capillary cone, or microbeaker is held to the oscillating arm, severe vibrations are set up in the contents of the glass apparatus, which have proved remarkably efficient in accelerating crystallization from supersaturated solutions<sup>9</sup> and in promoting flocculation of colloidal suspensions.

The little effort spent in assembling this commonplace device will be well repaid. It may be used to obtain, in a short time, complete separation of precipitates as magnesium-ammonium phosphate, magnesium-ammonium arsenate, potassium-hydrogen tartrate, sodium-zinc-uranyl acetate, ammonium phosphomolybdate, etc. The flocculation of sulfide precipitates can be speeded up considerably. This has proved very helpful in the precipitation of germanium disulfide, which has a definite tendency to give colloidal suspensions. The sulfides of molybdenum, arsenic, tin, lead, cadmium, and zinc as well as the halides of silver furnish some of the examples which invite use of the buzzer.

**STOPPERS AND RUBBER TUBING**

Rubber stoppers and rubber tubing used in the assembly of microchemical apparatus must be elastic and flexible. Protective coatings on the outside of stoppers must be removed. Tubing should be tested by cutting open a short section. The inside must present a smooth, clean surface of live rubber.

Rubber tubing of 3-mm bore and 2-mm wall thickness deserves the widest use for flexible connections of every kind. Because of the small bore and relatively thick wall, such tubing carries vacuum without collapsing. The customary type of heavy suction tube must be avoided. It is so stiff that it upsets most microchemical apparatus. The general employment of short pieces of tapered glass tubing *c*, Fig. 14A, is recommended wherever the 3-mm rubber tubing cannot be directly connected to wide glass tubing.

<sup>8</sup> Attention is called to an experiment with a violin string: J. Dunning and Ph. Pratt with O. E. Lowman, *J. Chem. Education*, 11, 624 (1934).

A small bottle with glycerin and a probing wire with cotton wads at both ends should be continuously ready for treatment of rubber tubing and stoppers. Assembly and the taking apart of apparatus are greatly facilitated if the rubber surfaces which make contact with glass are provided with an invisible film of glycerin.

### SUCTION AND PRESSURE DEVICES

Suction and pressure are best supplied by the customary water pumps available in every chemical laboratory. Apparatus must not be connected directly with the suction pump. Insertion of a trap gives sufficient protection against the consequences of failure of the

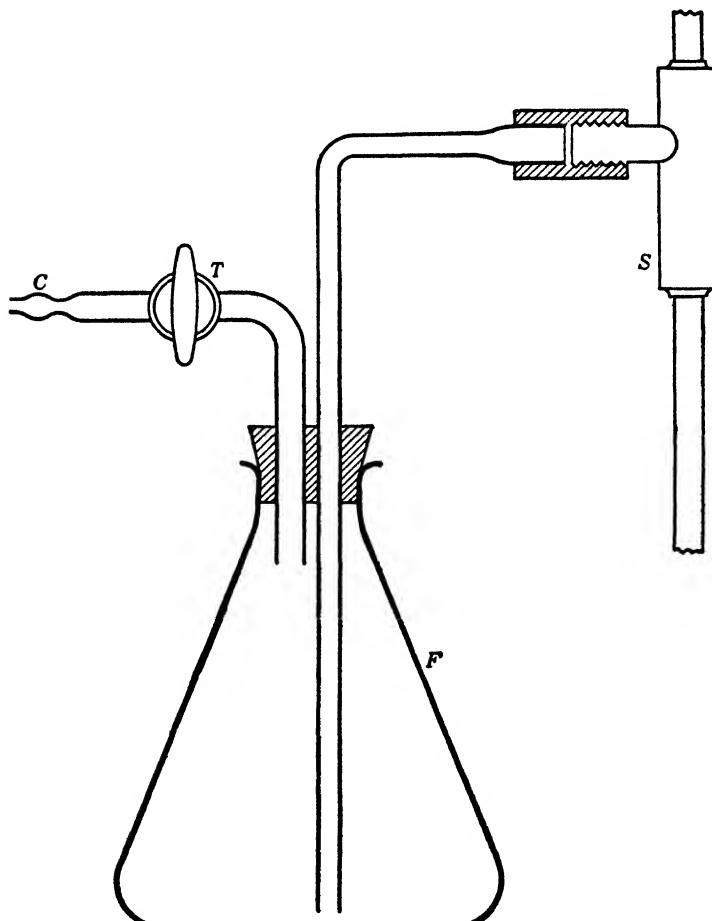


FIG. 12. Suction pump and trap.

pump. The trap shown in Fig. 12 requires a 250-ml filtering flask with heavy walls, a two-hole rubber stopper No. 6, and a three-way stopcock *T* with outlet through the bottom of the plug. The stopcock

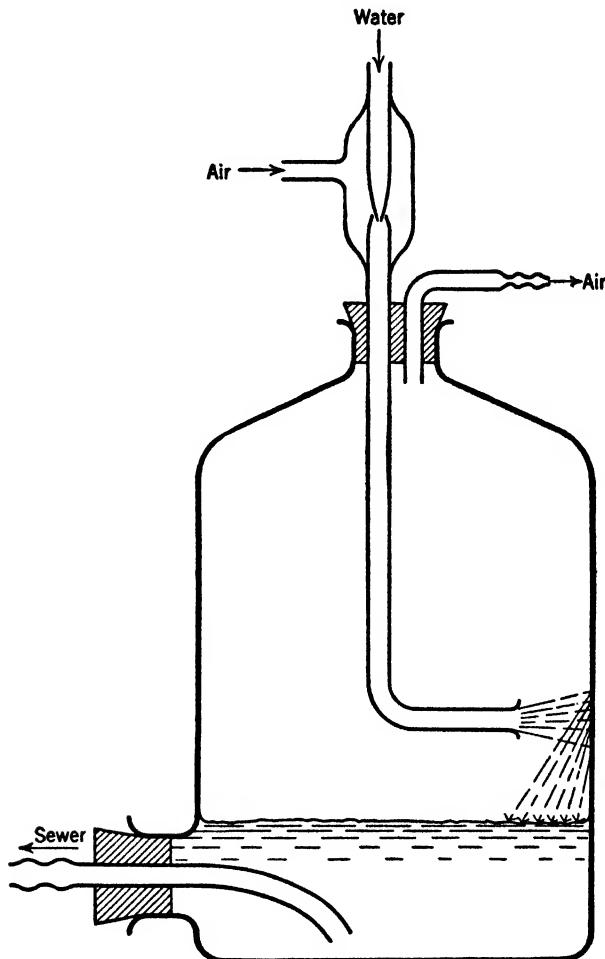


FIG. 13. Water-blower.

provides a satisfactory means for breaking the suction. The taper of tubing *C* facilitates the use of rubber tubing of 3-mm bore.

A water-blower, Fig. 13, is the most convenient source for clean air of light pressure. A suction pump and a 5-liter aspirator bottle are needed in its construction. As a satisfactory alternative, oxygen or nitrogen may be supplied through a reducing valve from a steel cylinder. One must beware, however, of pumps and blowers which

have the moving parts immersed in oil. The air delivered by such pumps often contains a considerable amount of organic vapors which are difficult to remove and which become the cause of annoying interferences in microchemical work.

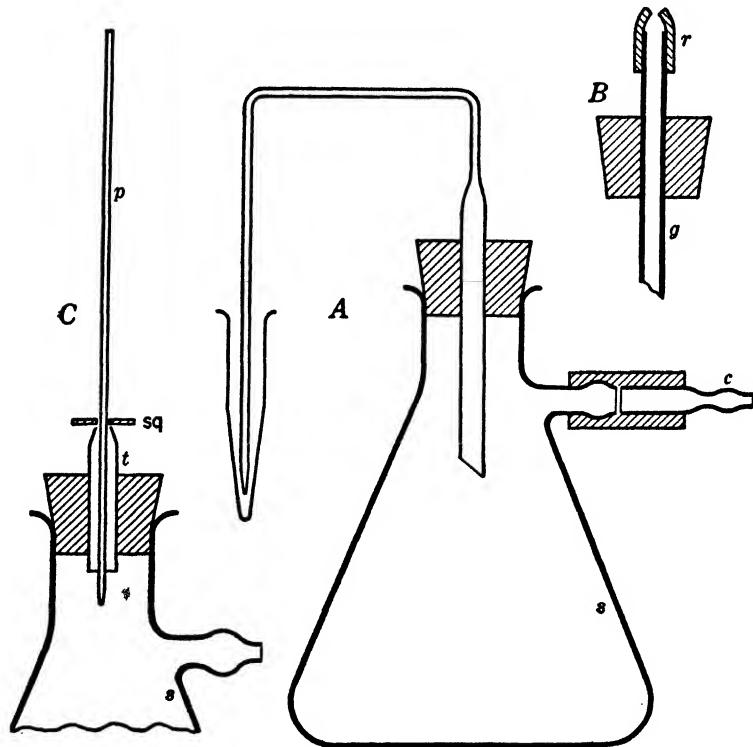


FIG. 14. Suction devices. *A*, rinsing of microcones; *B*, device for cleaning pipets; *C*, device for cleaning capillary pipets.

For the cleaning of various apparatus a 250-ml filtering flask with side arm, which should be provided with three one-hole rubber stoppers No. 5, is indispensable. The first stopper holds a capillary siphon, Fig. 14A, needed in the cleaning of centrifuge cones. The second stopper, Fig. 14B, is equipped with a piece of glass tubing, 6 mm in outside diameter, one side of which is fitted with a short piece of rubber tubing *r* of 2- to 3-mm bore. This device is very useful in the cleaning of thermometer capillaries, micropipets, and microburets. In the third rubber stopper is inserted a glass tube of 6-mm outer diameter which tapers on one side to an opening of 2 mm. Figure 14C shows the use of this gadget in the cleaning of capillaries.

The suction flask *s* is best permanently fitted with a short piece of tapered tubing *c*, if it is impossible to attach rubber tubing of 3-mm bore directly to the side arm.

Stopper (*C*) fits also the Pyrex test tube with side arm shown in Fig. 15. The tube is 25 mm in inside diameter and 140 mm long. The side arm of 4-mm outside diameter is attached near the bottom

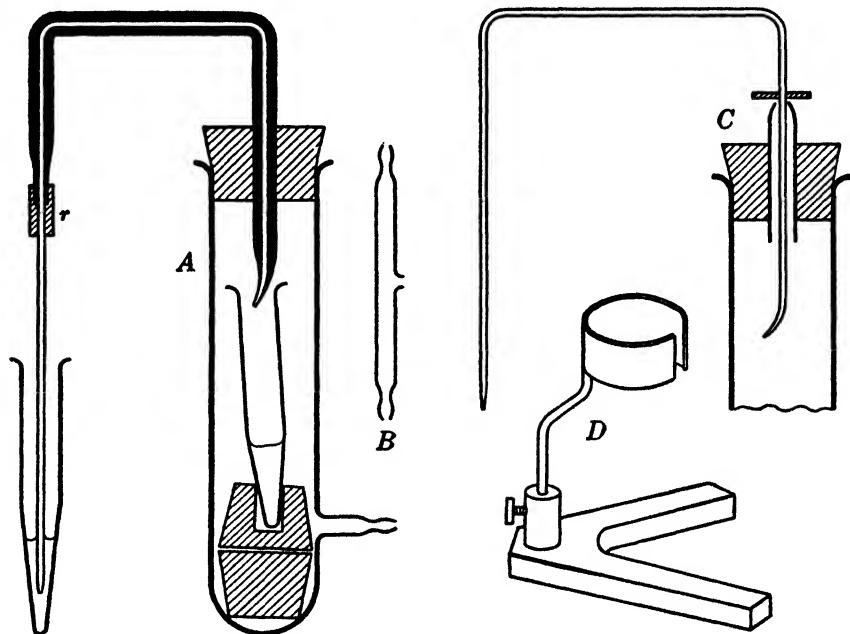


FIG. 15. Siphons operated by suction: *A*, using a thermometer capillary; *B*, tube for control of suction; *C*, capillary siphon; *D*, stand.

of the tube so that, when the vacuum is suddenly broken, the air rushing into the tube will not cause spattering of the liquids in the cones or beakers inside the suction tube. A one-hole rubber stopper No. 5 is permanently fitted with a siphon made of thermometer capillary, Fig. 15*A*. A short piece of soft rubber tubing *r* of 1-mm bore is used to connect capillary pipets or filtersticks. A tube *B* with a small opening in the middle may be inserted in the suction line. Especially when lifting centrifugates off precipitates, it is desirable to have excellent control over the functioning of the siphon. If a slight vacuum is maintained by closing the opening in the middle of tube *B* with the fingertip, it may be immediately broken by simply lifting the finger. A suitable stand for suction tubes is shown in Fig. 15*D*.

## EXPERIMENT 3

## Cleaning of Microcones

Microcones and centrifuge cones are cleaned by means of feathers of suitable size, using first tap water, then soap solution, again tap water, and finally distilled water. If the cleaning is performed immediately after use, a practice which is highly recommended, there will be no need for special solvents. The suction device, Fig. 14A, serves for the final rinsing with distilled water. The cone is filled with water and held so that the opening of the siphon is in the point of the cone. The cone is filled three or four times with distilled water from the wash bottle without being removed from the suction device. Microcones are finally dried by centrifuging briefly and removing the droplet of water, collected in the point, with the suction device. The larger centrifuge cones may be dried on the customary drain racks. The clean cones are kept in a closed cardboard box.

## THE MICROSCOPE

For information on the construction of the microscope and on the theory of the formation of the microscopic image the reader is referred to the special literature.<sup>10</sup> The microanalyst very rarely requires magnifications of more than 250, and, thus, the following suggestions on the use and care of the microscope are limited to the requirements of low-power microscopy.

The essential parts of a simple microscope are indicated in Fig. 16. A simple but rugged stand of standard size is desirable for use in microanalysis. Coarse adjustment with rack-and-pinion motion is very desirable, while fine adjustment is not required. A tilting mechanism is not essential. The stand should be provided with a combination plane and concave mirror which can be rotated around its axis and swung to the left and right by means of the suspension mounting of the frame. Condenser and diaphragm are superfluous in low-power microscopy. It is advisable, however, to have a ring provided below the stage which permits attachment of a standard condenser or a polarizer-condenser combination whenever desired. Two dry objectives having magnifications of 4 and 10 and a standard eyepiece giving a magnification of 8 provide the two most frequently required

<sup>10</sup> E. M. Chamot and C. W. Mason, *Handbook of Chemical Microscopy*, Vol. 1, 2nd ed., New York, John Wiley & Sons, 1938.

total magnifications of approximately 30 and 80. The use of expensive, highly corrected objectives is not justified, since, as a rule, the optical properties of the preparations of microanalysis deviate

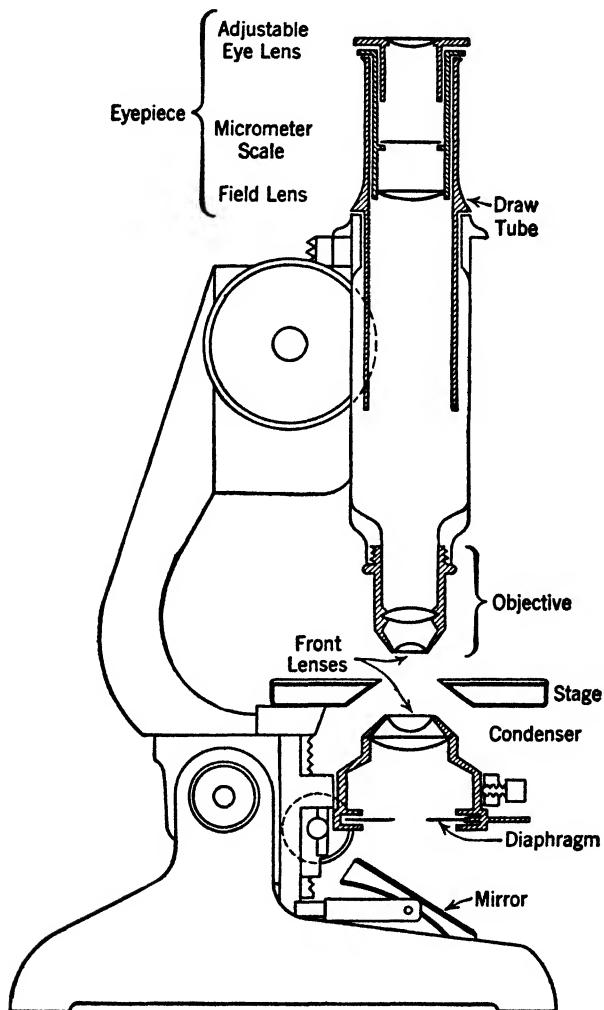


FIG. 16. Microscope.

considerably from those of biological preparations or polished specimens, for which the corrections of such lenses have been calculated.

The **total magnification** of the microscope is *equal to the product of the individual magnifications of objective and eyepiece*. Total magnifications of less than 100 are considered "low," and total mag-

nifications of more than 500 are classified as "high"; magnifications of 100 to 500 are called "medium."

The **objective** is the most important lens combination of the microscope, since the quality of the final image is largely determined by the proper functioning of the objective. It is imperative, therefore, to treat objectives with *great care*. When not in use, they must be enclosed in the metal containers supplied by the manufacturer.

Objectives usually consist of several lenses mounted in a metal tube, on which information concerning magnification, focal length  $f$ , and numerical aperture is engraved. The indicated magnification assumes the use of that tube length which is considered standard by the manufacturer of the objective (usually 160 mm, Leitz 170 mm).

$$\text{Magnification of objective} = \frac{\text{Tube length}}{\text{Focal length of objective}}$$

**Front lenses** are the lenses of the objective *and of the condenser* which are the closest to the preparation on the stage, Fig. 16. Their exposed surfaces are most likely to become soiled or mechanically damaged by the preparations, and they must be *inspected frequently*.

The **working distance** of the objective is the actual distance in millimeters between front lens and preparation when the object is in focus. The working distance of a low-power objective is somewhat smaller than its focal length. The latter may be used as a crude estimate of the actual working distance. It can be easily calculated, if the magnification of the objective is known. Knowledge of the approximate working distance is desirable, since it facilitates focusing.

**Angular aperture (A.A.)** of an objective is determined by the angle, indicated in Fig. 17A, between the most divergent rays used for image formation. The numerical aperture (N.A.) is given by the equation,

$$\text{N.A.} = n \cdot \sin \frac{\text{A.A.}}{2}$$

in which  $n$  is the refractive index of the medium filling the space between preparation and front lens of the objective.

The **eyepiece** shown in Fig. 16 is of the common, Huygensian type. The real image, produced by the combined action of objective and field lens of the eyepiece, forms in an image plane located about halfway between the two lenses of the eyepiece. When micrometer scales, cross hairs, or other rulings are placed in the eyepiece (Fig. 16), this image plane becomes fixed in a definite position, and the real image produced by the objective must be formed in this plane.

The eye lens is then able to produce simultaneously a sharp image of rulings and specimen. Since the rulings are in a fixed position, provision must be made to allow the focusing of the eye lens on the rulings by mounting the eye lens in a short tube which telescopes or screws into the top of the eyepiece, Fig. 16.

Eyepieces are commonly designated by their magnification, and the designation always appears on the top face of the mounting.

$$\text{Eyepiece magnification} = \frac{250}{\text{Focal length in millimeters}}$$

The eyepiece remains safely in the microscope as long as the *body tube is not inverted*. Eyepieces which are not in use should be kept in the rack provided in every microscope case. One eyepiece must always remain in the tube of the microscope, since otherwise dust would collect in the interior of the body tube and on the uppermost lens surface of the attached objective.

### MICROSCOPE LAMPS

A microscope lamp for microchemical use must permit quick adjustment in height so as to allow rapid interchange of transmitted and reflected light. It should permit focusing a small spot of light of high intensity on the object of investigation, and it should allow the insertion of ground-glass plate and color filters (daylight filters) singly and in pairs. Finally, such a microscope lamp should be light in weight, small in size, and it must not heat up to such an extent that it becomes difficult to handle. Obviously, a low-voltage bulb, consuming a few watts and mounted with a condenser lens in a telescoping tube, would approximate the requirements outlined above. The tube might be mounted in a well-balanced manner on a ball-and-socket universal joint, which in turn could be made adjustable along a vertical rod.

Most of the microscope lamps on the market are not suitable for microchemical work, and those models which possess satisfactory flexibility of adjustment are expensive. As a substitute, desk lamps with gooseneck arm are quite satisfactory. The adaptation of spot lamps for automobiles and motorcycles as well as of electric bicycle headlights may be considered. A rather efficient and very handy microscope lamp can be obtained with the use of Mazda Lens Bulb 222 (0.55-watt) for Pen Lights. The glass at the top of the tiny bulb is fused into a bead which acts as a powerful condenser lens. Potential of 2.2 volts may be supplied to several of these bulbs by means of an

ordinary 2.5-volt filament transformer and a 10-ohm variable rheostat in series with the bulbs.

#### EXPERIMENT 4

##### Cleaning and Inspecting the Microscope; Standard Procedure of Focusing

Lens paper.

Camel's-hair brush, round. Bathe in 1 ml of acetone to remove fat, and allow to dry.

Cotton and toothpicks.

Clean rag.

The microscope is removed from its case and placed on a clean table top. When carrying a microscope, its arm is grasped with the right hand and the *base* of the stand is *supported* with the left. This prevents accidents such as smashing the lower part of the microscope into the edge of a table top.

After the case has been inspected inside and outside, and the inventory checked for objectives, containers, eyepieces, racks, and other accessories, the following procedure is complied with:

1. The metal parts of the microscope, stand, stage, substage, and tube are examined and cleaned with a soft rag, if necessary.

2. Both sides of the mirror are cleaned with lens paper.

3. PRELIMINARY ADJUSTMENT OF ILLUMINATION. A lamp is placed in front of the microscope, and the concave mirror is adjusted until it reflects the light through the hole in the stage to the front lens of the objective. If the substage carries a diaphragm, it should be opened wide. In the absence of a microscope lamp, any source of light—preferably sunlight reflected from white clouds or white walls—may be substituted, but it will be necessary to determine whether the concave or the plane side of the mirror gives the stronger illumination.

4. A *clean* microscope slide is placed on the stage so that one of its long edges runs across the center of the hole in the stage.

5. STANDARD PROCEDURE OF FOCUSING. (a) The working distance of the attached objective is estimated from the data available. Information on focal lengths and magnifications is often posted inside the microscope case.

(b) The objective is observed from the *side*, the eyes being held on the level of the microscope stage, and the tube is lowered until the front lens of the objective has approached the preparation to approximately half of the estimated working distance.

(c) While the observer is looking into the eyepiece, the tube is slowly *raised* until a sharp image of the upper edge of the slide is obtained. No image will be obtained if either the front lens of the objective was originally above the working distance, or the slide is not in the proper position on the stage. After the necessary adjustments have been made the focusing is repeated as directed under (b) and (c).

6. FINAL ADJUSTMENT OF ILLUMINATION. The mirror is adjusted, trying the plane and the concave surface, until the most effective and even illumination of the whole field is secured. If the illumination is too strong, a ground-glass plate or a sheet of paper may be placed in front of the condenser lens of the lamp, or the lamp may be moved farther away from the microscope.

If the microscope has a condenser and a diaphragm, the condenser is moved up and down until the strongest possible illumination is obtained. The intensity of the illumination is finally adjusted by reducing the opening of the substage diaphragm.

7. EXAMINATION OF THE IMAGE. The edge of the slide should appear sharply defined, but otherwise the field should be empty. Films of dirt on the lenses of the microscope proper will impair the definition of the image; the edge of the slide will appear somewhat blurred or clouded by a haze, and refocusing effects no improvement. *Particles* adhering to lens surfaces are indicated by the presence of more or less sharply defined spots in the field of vision. The imperfections of the optical system are located as follows:

(a) The **slide** is moved. Images of dust particles (fingerprints) adhering to the slide move simultaneously.

(b) The **eyepiece** is rotated. This imparts a rotary motion to images produced by dust or dirt located on the lenses of the eyepiece.

(c) The mirror is slightly moved, or the condenser, if one is used, is focused up and down. Images of irregularities in the **light source** or images of foreign matter adhering to the **condenser** lenses are easily recognized. The former are eliminated by proper adjustment of the distance of the light source from the microscope; the latter, by cleaning the lenses of the condenser and the aperture of the substage diaphragm.

(d) If the imperfections are not located in either of the aforementioned parts of the optical system, then they must originate in the **objective**. Corrosion, dirt, and dust on the lenses of the objective may be recognized by the fact that they destroy the clarity and sharpness of the final image, while their own images are very vague or not even perceptible.

(e) Interfering images, which are sharp in focus simultaneously with the image of the edge of the slide, are obviously caused by objects located in one of the **conjugated image planes**: the plane of the field diaphragm of the eyepiece (micrometer scale), the preparation itself, the source of illumination, or some plane between condenser and lamp.

8. INSPECTION AND CLEANING OF EYEPieces. Before any cleaning of optical parts is attempted the hands should be carefully washed and dried. The eyepiece is taken out of the tube of the microscope, the mountings of eye lens and field lens are unscrewed, and the parts are placed on a clean piece of paper. The lens surfaces are inspected for corrosion, scratches, and dirt by holding them so that the light is reflected from them toward the eye. All lens surfaces should appear as clean, flawless mirrors. By rotating the lenses around their optic axes it is possible to distinguish between reflections and imperfections; imperfections rotate with the lenses, whereas reflections remain more or less stationary.

For cleaning, one breathes on the lens surfaces and then removes the moisture by means of double layers of lens paper. Not too much pressure should be applied, as it might cause the oil of the skin to penetrate the paper. All lens surfaces are finally inspected again, and, if the result is satisfactory, the eyepiece is assembled immediately.

MICROMETER EYEPieces unscrew in the middle to permit access to the plate bearing the rulings. When unscrewing, it is essential to hold the casing right side up so that the micrometer plate will not be dropped. The micrometer plate may be taken out of the tube, inspected, and cleaned as described for lenses. Often, however, it will be sufficient to leave it in the tube and to clean the upper surface of the micrometer plate with the camel's-hair brush.

CROSS HAIRS are often real hairs, mounted on a ring inside the eyepiece. Such hairs are easily broken, but cleaning is rarely necessary, since, because of the absence of a glass plate, dust cannot collect in the image plane. Cross hairs, which are engraved on a glass plate, are treated the same as micrometer plates.

9. CLEANING OBJECTIVES. The principle of the inspection of lens surfaces has been outlined in the preceding paragraph. *Standard objectives must not be taken apart*, and cleaning, which requires the separation of the lenses, is to be performed by the manufacturer. The cleaning is, therefore, limited to the lower surface of the front lens and the upper surface of the top lens. The latter surface is usually inside the tubular case of the objective. One uses a swab of cotton

on a toothpick for cleaning, and it is obvious that substitution of metal of any sort for the wood is not permissible. The wiping with cotton should be followed by dusting with a camel's-hair brush or strong blasting with a current of clean air.

10. INSPECTING THE COARSE ADJUSTMENT. Rack and pinion are tested for excess play; the body tube should remain in every position in which it is placed.

Many microscope stands still lack a stop preventing the pinion from overriding the rack and, consequently, the coarse adjustment is frequently damaged by inexperience, haste, or carelessness in meshing the gears. For inspection, the body tube is raised to its highest position with the use of the pinion head. It will be noticed that the gears disengage and rotation of the pinion head fails to raise the tube any higher. The top of the body tube is now grasped and removed from the stand. Rotation of the pinion head permits inspection of the teeth of the pinion. Any imperfections of these or of the teeth of the rack must be corrected immediately, since otherwise use will destroy the mechanism.

MESHING OF RACK AND PINION. The rack of the body tube is cautiously inserted in the pinion slot, and the tube is carefully pushed down until the rack touches the pinion. *Slight* motions, forward and backward, are now made with the pinion head, and very *gentle* pressure is applied to the tube until proper meshing takes place. The coarse focusing device must now work *smoothly*. The procedure is to be followed whenever it happens that the pinion overrides the rack.

11. CLEANING CONDENSERS. Condensers usually can be removed from the stand and taken apart. Most of the dust collects on the top surface of the front lens.

12. FINAL INSPECTION. After cleaning, the microscope is assembled, and the tests described under 7 are repeated. It will be seen whether it is necessary to repeat the cleaning process. Permanent imperfections, such as slight corrossions or scratches on the lenses, can be removed by the manufacturer.

## EXPERIMENT 5

### Illumination of Microscopic Specimens

Ground-glass slide.

Paper, black, white, and colored.

India ink, cedarwood oil.

A suitable microscopic specimen is obtained as follows. A line is drawn with a silver coin through the center of the rough surface

of a ground-glass slide. Parallel to this line and approximately 1 mm away from it, another streak is made with a penny. Finally, with a soft pencil or India ink, a short millimeter scale is laid at right angles across the two former lines. The millimeter scale can be easily copied by laying the slide on millimeter graph paper.

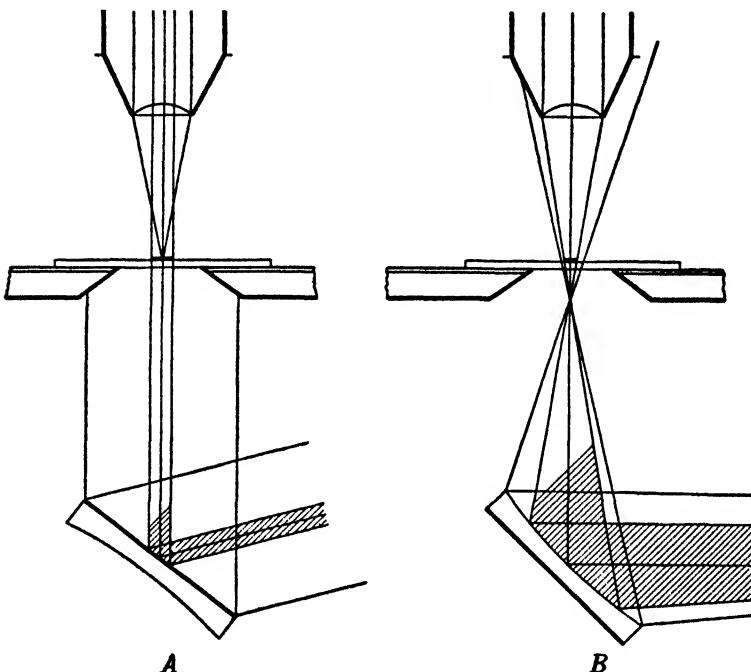


FIG. 17. Use of plane and concave mirrors. The diameters of the preparations and the angular apertures of the objectives are indicated. The shaded areas show the bundles of light which are used for the formation of the image.

**1. REFRACTIVE INDEX AND VISIBILITY.** The slide is placed on the stage of the microscope and the millimeter scale is focused, a total magnification of 50 to 80 being used and illumination being provided by light from the substage mirror. Focusing by the standard procedure and the adjustment of the illumination have been described in Expt. 4, items 5 and 6; the choice between plane and concave mirror is determined largely by the size of the field of vision and the required light intensity, Fig. 17. Observation through the eyepiece will finally show that the structure of the rough surface of the slide is distinctly visible and interferes greatly with the recognition of the particles left behind by pencil and coins.

The slide is now removed from the microscope and a large drop of cedarwood oil and then a cover slip are placed on the millimeter rulings. There should be enough cedarwood oil to fill the space between the slide and the cover slip completely. When the millimeter ruling is again focused under the microscope, it will be noticed that the disturbance by the rough surface of the slide has vanished and that it is difficult to discern the structure of the glass surface. This phenomenon is produced by the close identity of the refractive indices of the glass and the oil. It follows that chemical heterogeneity need not cause optical heterogeneity, which is determined by differences in refraction and absorption of light.<sup>11, 12</sup>

2. OBSERVATION WITH TRANSMITTED LIGHT. The slide is moved so that the streaks made with the coins and lines of the millimeter scale are simultaneously in the field of vision. It will be noticed that the particles of metal as well as those of graphite or ink appear black. To make certain that no light other than that coming from the mirror is used for illumination, the preparation is shielded by cupping the hands around the stage. With light coming exclusively from below the stage, it is obvious that *all opaque* materials must appear *black*, since the microscope views that side of the objects which is not illuminated by the mirror.

3. OBSERVATION WITH REFLECTED LIGHT. The microscope lamp is now raised above the level of the stage and the light is sent down directly on the preparation. The metal particles will now exhibit their characteristic colors, but it will be noticed that a background of suitable color is required to provide the necessary contrast for the simultaneous observation of the black lines, the silver particles, and the bronze streak. The most efficient background will be found by placing papers of different color (black, white, red, green, blue) underneath the slide. The preparation is to be saved for Expt. 6.

It need hardly be pointed out that, when working with artificial light, a daylight filter is advisable for a satisfactory identification of colors. Direct sunlight or the light of a carbon arc are ideal light sources for observation with reflected light.

The illumination of large objects follows the same principles. As a matter of fact, the whole Expt. 5 may be carried out without a microscope, and even the aid of a magnifying glass is not required.

<sup>11</sup> On the determination of refractive indices by the immersion method see E. M. Chamot and C. W. Mason, *Handbook of Chemical Microscopy*, 2nd ed., Vol. 1, New York, John Wiley & Sons, 1938.

<sup>12</sup> The observation of *schlieren* is discussed in F. Emich and F. Schneider, *Microchemical Laboratory Manual*, New York, John Wiley & Sons, 1932.

The principal methods of illumination employed with the microscope are listed in Table I, in which, according to common usage, a beam of light signifies a bundle of parallel rays and a pencil of light indicates a cone of convergent or divergent rays.

TABLE I  
METHODS OF ILLUMINATION USED IN MICROSCOPY

Observation with Transmitted Light	Observation with Reflected Light
<b>Axial:</b> (a) <i>Parallel:</i> beam of light parallel to the vertical axis of the microscope; <i>source of light at a great distance, plane mirror situated in the axis of the microscope</i> (Fig. 17A).	<b>Axial:</b> beam or pencil of light parallel to the optic axis; <i>placing transparent reflector (i.e., cover slip) between the objective and the specimen so that a horizontal beam of light is reflected parallel to the axis of the microscope onto the surface under examination; vertical illuminator for medium- and high-power objectives (metallography).</i>
(b) <i>Convergent:</i> pencil of rays along the vertical axis of the microscope; <i>concave mirror or condenser</i> (Fig. 17B).	<b>Inclined:</b> beam or pencil of rays inclined to the optic axis; <i>direct illumination from light source located above the stage.</i>
<b>Oblique:</b> (a) <i>Parallel:</i> beam of light inclined to the axis of the microscope; <i>plane mirror swung to one side.</i>	<b>Annular oblique:</b> <i>Silverman illuminator, Epi-Condenser W (Zeiss), Ultropak (Leitz).</i>
(b) <i>Convergent:</i> pencil of rays inclined to the vertical axis of the microscope; <i>concave mirror swung to one side; a condenser screened on all sides except one; or a condenser with the small opening of its iris diaphragm off center.</i>	
<b>Annular oblique:</b> <i>Dark-field condenser.</i>	

## EXPERIMENT 6

### Calibration of Eyepiece Micrometer Working Distance and Field of Vision of Objective

Stage micrometer or  
Ground-glass slide used in Expt. 5.

The value of a division of the eyepiece micrometer scale depends upon the magnification of the image produced in the plane of the micrometer scale, i.e., it depends on the magnifying power of the objective and on the tube length. Accordingly, the eyepiece micrometer scale must be calibrated separately for every objective and every *tube length* employed in conjunction with the micrometer eyepiece. If the draw tube of the microscope has a scale engraved, the tube length may be adjusted as desired, preferably to the standard tube length for which the objective is designed. When working with a simple microscope which does not have graduations on the draw tube, it is best to use the minimum tube length, since it can be reliably reproduced.

**Stage micrometers** consist of a glass slide with an accurately divided scale in the center. The scale is protected by a cover slip. The value of the divisions is usually given on a label attached to one end of the slide. The stage micrometer may be cleaned by wiping with lens paper, but *special care* must be taken not to damage or remove the cover slip protecting the scale.

**PROCEDURE.** First of all the tube length is adjusted according to the decision on this point. Then the micrometer eyepiece is removed from the microscope and the eye lens is focused sharply on the micrometer scale while the observer looks through the eyepiece toward a brightly illuminated object (white paper, lamp). Finally, the micrometer eyepiece is inserted into the microscope *without changing* the focus of the eye lens.

After it has been made certain that the proper objective has been attached to the microscope, the stage micrometer is placed on the stage of the microscope so that the *cover glass is on the upper side* facing the objective.

The direct focusing of the micrometer scale is rather trying, since it is troublesome to adjust the slide so that the very small scale, which can be seen only with difficulty, appears in the field of vision. It is preferable to focus the microscope in a preliminary way on some coarse structure located in approximately the same level as the object in question. The edge of the label or the upper edge of the slide are suitable reference marks in the present instance, and either one of them is focused by the standard procedure. Then the stage micrometer is moved until the scale appears in the field of vision, whereupon the position of the microscope is readjusted until a perfectly sharp image of the stage micrometer scale is obtained. By moving the slide and rotating the eyepiece, both scales are now aligned so that they partially overlap as shown in Fig. 18.

**TEST FOR ABSENCE OF PARALLAX.** If all the focusing has been done properly, a movement of the eye in front of the eyepiece will cause no relative displacement of the images of the two scales, and no parallactic error can enter the calibration. If, however, the scales seem to change their relative positions when the eye is moved, the focusing of both micrometer scales must be repeated. It may be helpful to

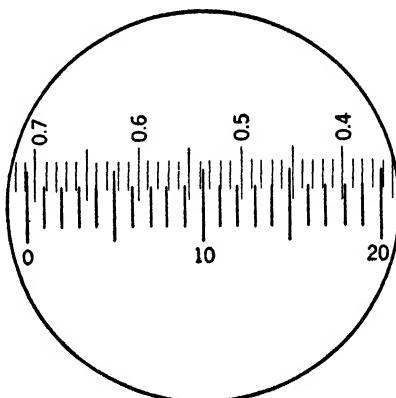


FIG. 18. Calibration of eyepiece micrometer.

mention in this connection that all microscopic work should be done with *completely relaxed* eye. Beginners should rest their eyes frequently by looking at a distant object. The use of the accommodating power of the eye not only harms the organ but also makes focusing uncertain.

**EVALUATION OF THE EYEPIECE MICROMETER.** Two points, located not too far from the center of the field and approximately one-third of the diameter of the field apart, are chosen where lines of the eyepiece scale and the stage scale coincide. If necessary, the stage micrometer is moved until desirable locations of coincidence are obtained. By counting, the distance between the points of coincidence is determined in units of both scales. The value of the divisions of the stage scale is known, and, thus, the actual distance between the two points of coincidence can immediately be expressed in microns. Division of this quantity by the corresponding number of scale divisions of the eyepiece micrometer gives the value in microns of one division of the latter scale.

**DIAMETER OF THE FIELD OF VISION.** The actual diameter of the microscopic field can be read directly off the stage micrometer scale. In the example of Fig. 18 it is 0.38 mm. The diameter of the field of vision depends mainly on the magnification of the objective; the choice of eyepiece is of little influence.

**WORKING DISTANCE OF OBJECTIVE.** The working distance is estimated with the help of a millimeter ruler held parallel to the optic axis of the microscope.

It is highly desirable to tabulate all data concerning the objectives, as value of the unit of the eyepiece micrometer scale, working distance, and diameter of the field of vision, and to post them inside the microscope case.

## EXPERIMENT 7

### Preparation of Capillary Pipets

A piece of capillary tubing of 0.5- to 1-mm bore and approximately 20-cm length is selected. The center of the capillary is heated in the edge of the lowest part of the Bunsen flame until the glass just softens. The capillary is then removed from the flame and the heated part is pulled out with a quick motion so that a fine capillary of 0.05- to 0.1-mm bore and approximately 10- to 20-cm length results. If the fine capillary can be bent into a loop without breaking, it probably has the desired diameter.

The fine capillary is broken at a distance of 20 mm from the tapered portions so that two capillary pipets are obtained, Fig. 19. Beginners are advised to inspect the fine capillary under the microscope, using a magnification of approximately 30, and to measure the bore of the fine capillary with the eyepiece micrometer. The capillary is laid on a slide and the slide is moved until the fine capillary is brought into the field of vision. It should be understood that this method for the determination of the bore gives only a crude estimate; an exact determination requires use of the method described in the following experiment.

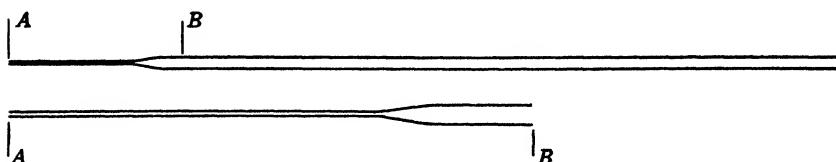


FIG. 19. Capillary pipet, approximately natural size. The tip is shown three times enlarged.

Preparation of eight or ten capillary pipets will suffice for the acquisition of the technique.

**USE OF CAPILLARY PIPETS.** When the tip of a capillary pipet is inserted in a liquid, the pipet begins to fill automatically because of the phenomenon of capillary attraction. The level to which the liquid rises is a function of the radius  $r$  of the bore of the capillary at this level and of the density  $d$  and the surface tension  $\gamma$  of the liquid,

$$h = \frac{\gamma}{494rd} \text{ cm}$$

The height  $h$  and the radius  $r$  are measured in centimeters. The surface tension  $\gamma$  has a value of approximately 72 dynes per centimeter for water and most dilute aqueous solutions of electrolytes at room temperature.

The *length* of capillary filled by a liquid depends essentially upon the inclination of the capillary to the horizontal; if  $\alpha$  is the angle of inclination, the length  $l$  is given by,

$$l = \frac{\gamma}{494rd \sin \alpha} \text{ cm}$$

The length  $l$  is equal to  $h$  if the pipet is in a perpendicular position, and it becomes infinity with the pipet in a horizontal position.

The *rate* at which the liquid enters is determined by the bore and shape of the tip of the pipet and by the viscosity of the liquid. Pipets with long, fine tips are best suited for measuring small volumes of liquid because of the slow rate of filling and delivery. Whenever necessary, the speed of operation may be increased and adjusted by breaking off portions of the tip until it possesses the desired opening. With a given pipet or capillary, the rise of liquid is controlled by properly inclining the tube and by withdrawing it when the meniscus has reached the desired position. Closing of the upper opening of the pipet with the finger does not give the needed control.

### EXPERIMENT 8

#### Preparation of Calibrated Capillary Pipets, Determination of the Bore of a Capillary

Millimeter rule.

Capillary clamp or substitute.

Micrometer eyepiece.

Square of sheet rubber.

If a suitable microscope is not available, capillary pipets may be calibrated by measuring and weighing a column of water as outlined in Expt. 9.

Calibrated capillary pipets are used for measuring volumes ranging from 1 to 20  $\lambda$ . A capillary of 0.5-mm bore and 12- to 15-cm length will possess the required capacity. Sufficient uniformity of the bore eliminates necessity for a tedious calibration of the various sections of the pipet.

A piece of capillary of apparently uniform bore of approximately 0.5 mm is selected, and a piece 15 cm long is cut off, great care being taken to obtain an even cut on both sides. For the *exact measurement of the bore* the capillary is mounted in the axis of the microscope so that it is possible to focus one of the end surfaces obtained by cutting. Since the capillary is too long to be placed on the stage, it is necessary to remove the condenser of the microscope and to swing the mirror aside. Then the capillary may be mounted on the table top in a vertical position so that its upper end projects through the center of the hole in the stage. The tube of the microscope should be raised out of the way, and special care must be taken not to scratch the front lens of the objective with the sharp end of the capillary. The capillary is held by a suitable capillary clamp,<sup>18</sup> or attached with Plasticine to the side of a rectangular block which is placed on the

<sup>18</sup> H. K. Alber, *Mikrochemie*, 14, 223 and 227 (1933); F. Emich and F. Schneider, *Microchemical Laboratory Manual*, New York, John Wiley & Sons, 1932, p. 93.

table top underneath the stage. The light of the microscope lamp is directed on the upper end of the capillary, and the top surface (cross section) is focused by the standard procedure. A magnification of 30 is quite satisfactory.

The microscopic image reveals the shape of the cross section of the capillary. The bore should be circular in outline; if any flattening is perceptible, the capillary is not suited for calibration. For the measurement of the diameter of the bore, the position of the capillary is adjusted and the eyepiece rotated until the dark circular disk which represents the image of the bore is properly aligned with the micrometer scale of the eyepiece. The length of the diameter is recorded. Then the capillary is turned upside down and the diameter of the bore is determined at the other end. If the two measurements agree within 10 per cent, the uniformity of the bore is considered satisfactory for use in approximate estimations, and one end of the capillary is drawn out to a fine point as described in Expt. 7. Very little of the length of the capillary will be lost if a piece of glass, to be used as a handle for the drawing of the tip, is first sealed to the end of the calibrated capillary. The resulting fine capillary is broken 1 mm from the end of the tapered portion. Of course, length and fineness of the tip determine accuracy, ease, and speed of operation of the capillary pipet and should be adjusted according to requirements and individual preference.

The result of the calibration is expressed as length  $l$  of the capillary which corresponds to  $1 \lambda$  capacity. This quantity is calculated with 2 significant figures from the equation

$$l = \frac{1}{\pi r^2} \text{ mm}$$

The radius of the bore  $r$  in millimeters is calculated from the average diameter of the bore. The calibrated pipet is best kept in a stoppered test tube, the label of which states the length  $l$ .

**USE OF CAPILLARY PIPETS.** Sufficient accuracy is obtained by measuring the length of the column of liquid with a millimeter ruler as shown in Fig. 20. Reasonable allowance should be made for the tapered part of the tip, but a high degree of accuracy is not necessary, since considerable uncertainty was introduced by calibrating the pipet for capacity instead of delivery.

**CLEANING OF CALIBRATED PIPETS.** Figure 20 shows the capillary fitted with a small piece of rubber sheet, approximately 1 cm square, which is cut from thin-walled rubber tubing as used with Gooch crucibles. The center of the square is punctured with a steel needle be-

fore the tip of the pipet is inserted. For cleaning, the capillary is mounted as shown in Fig. 14C. When suction is applied the rubber square produces a tight seal and the air is sucked through the capillary pipet. Cleaning solution may be transferred dropwise by means of a glass rod to the wide opening of the pipet and is quickly sucked

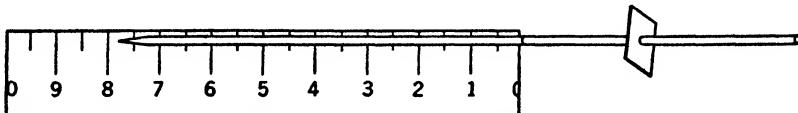


FIG. 20. Use of calibrated capillary pipet.

through the capillary. Washing with water is simply accomplished by touching the wide opening of the pipet with the nozzle of the wash bottle. Finally, the rubber square is lifted off, and, without removing the pipet altogether, some water is applied to the tapered tube of the suction device. After this treatment the rubber square is again allowed to form the seal, the pipet is rinsed once more, and is finally dried by sucking air through it for one minute.

## EXPERIMENT 9

### Preparation of Centrifugal Pipets

Analytical balance; set of weights.

Centrifugal pipets have the advantage of causing no loss of material, for the film of liquid which remains behind when ordinary pipets are emptied is collected by means of centrifugal force. Figure 21 shows a centrifugal pipet resting in the opening of a microcone. When this combination is whirled in a centrifuge, all the liquid is collected in the point of the cone.

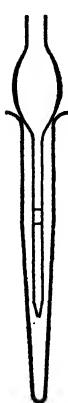


FIG. 21.  
Centrifugal  
pipet in  
microcone.

Centrifugal pipets of  $10\text{-}\lambda$  delivery may be easily prepared from suitable bulb-shaped pieces obtained when drawing capillaries. The fine tip of the capillary is made as usual. The calibration is most quickly performed with an analytical balance. The empty pipet is counterbalanced to the nearest tenth of a milligram. Then 10 mg is added to the other side of the balance and the final counterbalancing is done by taking water, a small amount at a time, into the pipet. When a drop of water is touched with the tip of the pipet, the water enters by capillary action. It enters the pipet very slowly, if the

point is sufficiently fine. An excess of water is easily removed by touching the tip of the pipet to a piece of filter paper. A large excess of water would cause an error since, on withdrawal of the water, a film of it would remain on the walls of the capillary. Any blowing with the mouth is not permissible in the course of calibration, and, obviously, the pipet should be clean to begin with and must be handled with clean, dry fingers. Rubber cots, worn on thumb and index finger of one hand, should be used in hot weather. It is understood that no water should adhere to the outside of the pipet and that the pipet must be placed on the pan of the balance so that the tip does not touch any part of the balance; a suitable rack may be bent of fine aluminum wire. When the desired quantity of water fills the tip and the wide part of the capillary as an unbroken column, the position of the meniscus is marked with a small strip of gummed paper which is later coated with collodion. As an alternative, one may measure the distance between meniscus and point of the tip and record it on the label of a small test tube used for storing the pipet between two plugs of cotton. The device shown in Fig. 14B is suitable for the cleaning of centrifugal pipets.

## EXPERIMENT 10

### Preparation and Calibration of Platinum Loops

4 cm platinum wire, B. & S. 30.

Calibrated capillary pipet.

Vise, steel needle, or paper clip.

One end of a platinum wire, 2 cm long and 0.3 mm in diameter, is bent around a steel needle 1 mm in diameter (paper clip), which is held in a vise. Flat-tipped forceps are useful in forcing the end of the platinum wire around the steel needle. The loop must lie in a plane, and it must be completely closed as indicated by Fig. 22a. The other end of the platinum wire is sealed into a glass capillary drawn out from a glass tube, Fig. 22. It is practical to bend the wire finally close to the loop so that the plane of the loop is inclined at an angle of 30 degrees to the rest of the wire,<sup>14</sup> Fig. 22b. Furthermore, it has been suggested that the loop be soldered closed with gold;<sup>15</sup> this is not necessary, however, if the loop is handled with care. It is obvious

<sup>14</sup> M. N. Short, "Microscopic Determination of the Ore Minerals," *U. S. Geol. Survey, Bulletin* 825, p. 52.

<sup>15</sup> F. Emich and F. Schneider, *Microchemical Laboratory Manual*, New York, John Wiley & Sons, 1932, p. 22.

that the sizes of wire and loop may be varied according to desire; a loop of the above dimensions holds approximately  $0.3 \lambda$  of aqueous solution.

**CLEANING PLATINUM LOOPS.** The freshly prepared loop is cleaned by placing it for a few minutes in concentrated hydrochloric acid, rinsing it with tap water, dipping it in distilled water, and finally igniting it in a non-luminous Bunsen flame. It is necessary to repeat this cleaning procedure every time the loop is used. A stream of clean tap water is conveniently supplied by allowing tap water to overflow into the sink from one opening of a U-tube, the other end of which

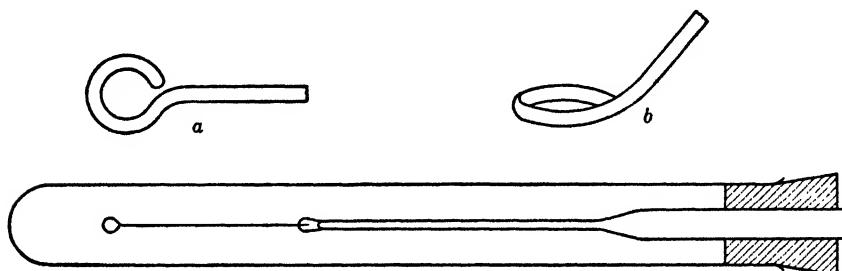


FIG. 22. Platinum loop; *a* and *b* approximately six times natural size.

is connected to a faucet.<sup>16</sup> Concentrated hydrochloric acid and distilled water are held ready in small bottles.

**CALIBRATION OF PLATINUM LOOPS.** A beaker of tap water and the calibrated capillary pipet are required. The clean loop is dipped in the water and withdrawn with a *slow* motion. During withdrawal, the loop must lie in a plane *perpendicular* to the surface of the water. The water taken up by the loop is quickly transferred to the calibrated pipet by touching the drop in the loop with the point of the pipet. The liquid is sucked automatically into the pipet and the volume is measured with the aid of the millimeter ruler. The water is then removed from the capillary pipet by touching the point to filter paper, the loop is ignited, and the calibration is repeated. It takes only a few minutes to carry out five determinations; the results are averaged and the capacity is calculated from the arithmetical mean of the five calibrations.

A particular loop was found to deliver  $0.75 \lambda$  when slowly withdrawn in a perpendicular plane, and  $1.2 \lambda$  when slowly withdrawn in a horizontal plane. If the loop was withdrawn rapidly in either position, larger volumes of liquid were delivered and the quantities of water taken up by the loop showed a wide variation.

<sup>16</sup> F. Emich, *Lehrbuch der Mikrochemie*, Wiesbaden, J. F. Bergmann, 1911, p. 62.

*PART II*



## QUALITATIVE ANALYSIS

### A. CONFIRMATORY TESTS

Chemical reactions cannot be observed. The objects of observation are the physical properties of the system before, during, and after reaction. Consequently, for the purpose of identification, the test material must be subjected to some treatment so as to produce characteristic physical phenomena which can be perceived by sense organs.

Most of the knowledge pertaining to the physical properties of matter is obtained by means of visual observations. Even when dealing with large quantities, close to 90 per cent of this information is observed either directly or indirectly with the eyes. When dealing with objects of microscopic dimensions, the senses of touch and taste become nearly useless. Considering that the olfactory effects of small quantities of matter cannot be intensified and that auditory perceptions are of very little use in chemical analysis, it follows that the importance of instruments for the magnification of optical appearances gains in proportion to the reduction of mass.

The majority of chemical confirmatory tests produce either one or both of the two following effects:

(a) Changes affecting the transmission of light by the appearance or disappearance of phases (boundary lines); changes from homogeneity to heterogeneity, and vice versa. In this category belong precipitation, evolution of gas, solution, condensation, evaporation, transitions to a different state of aggregation, etc.

(b) Distinct changes affecting the absorption of light, i.e., changes of color.

Both classes of phenomena allow direct visual perception on a large scale. With the aid of a microscope, they may be observed on objects less than  $1 \mu$  in diameter and less than  $10^{-12}$  ml in volume.

The practical value of any analytical procedure depends mainly upon its selectivity and its limiting proportions.

The degree of selectivity of a test, a method of separation, or a reagent is determined by the number of substances responding in a like manner. A test which is given by only a small number of sub-

stances is called **selective**.<sup>1</sup> Tests (procedures, reagents) to which only one substance responds are classified as **specific**.<sup>1</sup>

The sensitivity of a test (procedure or reagent) is to be described<sup>2</sup> precisely by listing the limiting concentration,<sup>2</sup> the limit of identification,<sup>2</sup> and the limiting proportions.<sup>3</sup> The **limiting concentration** (L.C.) is defined as the lowest concentration of substance (ion) which always produces a positive test. Its dimension is mass per volume. The **limit of identification** (L.I.) is the smallest *absolute* quantity (mass or volume) which always gives a positive test.

The **limiting proportions** (L.P.) are the smallest ratios

$$\frac{\text{Mass of substance sought for}}{\text{Mass of interfering matter simultaneously present}}$$

in which the sought-for substance is still detectable, provided that such an amount of mixture is used that the quantity of sought-for substance is equal to, or exceeds, the limit of identification. Limiting proportions are of paramount importance in analytical separations and in the interpretation of the results of analyses, as well as in the evaluation of confirmatory tests. As a rule, the limiting proportions are far less favorable than the limiting concentrations or the limits of identification which apply only after the substance has been isolated in a more or less pure state.

The limiting proportions are determined by essentially chemical phenomena as solubility, slow rate of reaction, coprecipitation, post precipitation, establishment of metastable equilibria, complex formation, and catalysis. Thus, it is reasonable to expect that the **absolute** quantities of matter will have little or no influence upon the limiting proportions. This assumption, which is of special importance in microanalysis, is supported by the available evidence, and it can be expected that limiting proportions, determined for milligram samples, will hold equally when working with one thousand or one million times smaller or larger quantities of material. In spite of the importance of the limiting proportions and their general usefulness, much valuable information is still lacking. This is partly because the determination of the large number of critical ratios requires a considerable amount of work, and partly because of lack of general recognition of the fundamental importance of the limiting proportions.

<sup>1</sup> Decision of the Committee for the Study of New Analytical Reagents, appointed by the International Union of Chemistry, Paris, May, 1937.

<sup>2</sup> F. Feigl, *Mikrochemie*, 1, 4 (1923).

<sup>3</sup> N. Schoorl, *Z. anal. Chem.*, 46, 658 (1907).

The limiting concentrations and the limits of identification are somewhat related, and both depend essentially upon the conditions under which the test is carried out and upon the methods used in the observation of the outcome. It is well known that, even in working with large samples, the recognition of slight opalescence or weak colorations depends mainly upon the proper methods of illumination and observation. If the efficiency of observation can be maintained constant, the limiting concentrations, which otherwise depend mainly upon chemical factors, are little affected by the scale on which tests are carried out. The limits of identification, however, improve when the tests are carried out with smaller volumes of solutions. As a matter of common sense, microchemistry has been developed to this very end, and the minima of mass which can serve for identification or determination are appropriate criteria of its progress. As to be expected, the limits of identification improve approximately in proportion to the reduction of the volume of solutions until finally, with extremely small volumes of solutions ( $10^{-10}$  ml), the methods for the demonstration of the outcome of tests gain primary importance, and the sensitivity of the tests is essentially determined by the limits of perceptibility.\*

Limiting concentrations, limits of identification, and limiting proportions cannot be determined with a high degree of accuracy because of the appearance of various uncertainties when the critical region is reached. For example, the perception of faint colorations depends very much upon the sensitivity of the eyes, which undergoes considerable variation even for one observer. The separation of precipitates becomes a matter of chance, when passing slightly below the limiting concentration. Taking  $40\lambda$  of 0.05 per cent magnesium sulfate solution for slide tests and adding an equal volume of  $\text{NaNH}_4\text{HPO}_4 \cdot 4\text{H}_2\text{O}$  solution, Richter<sup>5</sup> observed the separation of crystals of magnesium-ammonium phosphate hexahydrate in 40 trials. When 0.005 per cent magnesium sulfate was taken, the test was positive in 24 experiments and negative in 16 trials. Taking 0.001 per cent magnesium sulfate, Richter obtained 6 positive tests in 40 trials. With 0.0005 per cent solution consistently negative results were observed. The conclusion is obvious. The conditions which always produce a positive result

\* A. A. Benedetti-Pichler and J. R. Rachele, *Ind. Eng. Chem., Anal. Ed.*, 12, 233 (1940).

<sup>5</sup> O. Richter, *Sitzber. Akad. Wiss. Wien, Math.-naturw. Klasse, Abt. I*, 111, 171 (1902). F. Emich, *Lehrbuch der Mikrochemie*, 2nd ed., Munich, J. F. Bergmann, 1928, p. 3. T. B. Smith, *Analytical Processes, A Physio-Chemical Interpretation*, 2nd ed., London, E. Arnold & Co., 1940, p. 346.

and those which always give a negative test have no sharp borderline. They are separated by a range of conditions, which produces uncertainty as to the outcome. This implies that the limiting conditions which "always" lead to positive results must be established by a sufficient number of experiments.

Concerning the interpretation of the results of confirmatory tests, it is well known that positive tests must be supported by blank tests with the reagents. It is equally important to realize that negative results must be interpreted in the light of the limiting proportions, limiting concentrations, and limits of identification of the test and of all operations preceding it. If the addition of ammonium oxalate and ammonia to tap water does not produce a turbidity, it is proved that less than 4 mg calcium per liter,<sup>6</sup> if any, is present. If, however, the same test is applied to a solution of magnesium nitrate containing 10 grams of magnesium ion per liter, the interpretation of a negative result must be based upon the limiting proportion <sup>7</sup> Ca : Mg = 1 : 100 rather than on the limiting concentration, and only concentrations of calcium ion equal to and higher than 0.1 gram per liter are excluded. It is obvious that, in an exact sense, the absence of a substance cannot be proved by the chemical tests in use at the present time.

### SPOT TESTS

The performance of spot tests is extremely simple and does not require special apparatus. A drop of the test solution is placed on reagent paper (litmus and turmeric paper are well-known varieties), or drops of test solution and reagent solution are brought together on filter paper, on a watch glass, or on a drop test plate (side test for excess ferrocyanide in the titration of zinc). The application to qualitative analysis received special attention when Feigl<sup>8</sup> demonstrated the remarkable limits of identification obtainable. The use of paper as a medium for chemical tests offers unique possibilities for the separation of substances on the one hand<sup>9</sup> and for the accumulation and

<sup>6</sup> R. K. McAlpine and B. A. Soule, *Prescott and Johnson's Qualitative Chemical Analysis*, New York, Van Nostrand, 1933.

<sup>7</sup> A. Souchay and E. Lenssen, *Ann.*, 100, 323 (1856); Th. Scheerer, *ibid.*, 110, 237 (1859).

<sup>8</sup> F. Feigl and Rosa Stern, *Z. anal. Chem.*, 60, 1 (1921); F. Feigl and J. W. Matthews, *Qualitative Analysis by Spot Tests*, 2nd ed., New York, Nordemann, 1939.

<sup>9</sup> F. Schönbein, *Ann.*, 114, 275 (1861); Th. Bayley, *J. Chem. Soc.*, 33, I, 304 (1878); Fr. Goppelsroeder, *Die Kapillaranalyse*, Dresden, Th. Steinkopf, 1910.

concentration of substances on the other.<sup>10</sup> A technique for further improvement of the limits of identification has been described by Clarke and Hermance,<sup>10</sup> who also advocate the use of test papers impregnated with difficultly soluble reagents. Test papers of this kind are preferred by H. Yagoda<sup>11</sup> for semi-quantitative estimations with the use of spot tests.

It is obvious that tests based on the formation of strongly colored compounds offer great advantages when paper is used as a medium. Thus, the use of organic reagents is in great favor. Some of these produce inner complex compounds with inorganic ions. Others produce characteristically colored lakes, and quite a few of them may be classified as redox indicators. The tests described on the following pages have been selected for their specificity rather than for their sensitivity. The tests are performed either on spot paper (drop test paper No. 601 of Schleicher and Schüll) or on quantitative filter paper No. 589<sup>3</sup> (blue ribbon) of the same firm. As a rule, the efficiency of the tests is greatly affected by the type of paper used. Aside from the paper and capillary pipets, no apparatus is required.

## EXPERIMENT 11

### Mercuric Iodide

L.I., 0.5 γ Hg

By means of a capillary pipet approximately  $1 \lambda$  of mercuric test solution (10 mg Hg per ml) is transferred to potassium iodide paper (Appendix). An orange-red spot forms around the point where the tip of the pipet touches the paper. When 0.1 per cent mercuric solution is used, the center of the spot remains colorless and the mercuric iodide appears in a ring zone. The color is judged best when the test has completely dried.

The red spot is finally treated with a drop of freshly prepared stannite reagent (Appendix). A change of color from red to black or gray confirms the presence of mercury.

Lead produces a yellow spot of lead iodide, which disappears on addition of stannite reagent. The pale yellow silver iodide is reduced to black metallic silver by stannite. Bismuth solutions produce a brownish black spot on iodide paper, which turns black with stannite

<sup>10</sup> B. L. Clarke and H. W. Hermance, *Ind. Eng. Chem., Anal. Ed.*, **9**, 292 (1937); **10**, 591 (1938); *Mikrochemie*, **21**, 300 (1937).

<sup>11</sup> H. Yagoda, *Ind. Eng. Chem., Anal. Ed.*, **9**, 79 (1937); *Mikrochemie*, **24**, 117 (1938).

reagent, and the blue spots produced by cupric ion or stannic ion fade on addition of stannite. Cadmium, antimony, and arsenic solutions do not give visible reaction products on potassium iodide paper.

### EXPERIMENT 12

#### Silver Chromate

L.I., 0.2 γ Ag

By means of a glass rod, drops of 2 per cent ammonium chromate solution are placed on spot paper. The paper is laid aside for 5 minutes to dry.

Approximately 1 λ of silver test solution (10 mg Ag per ml) is taken up in a capillary pipet. If too much of the solution enters the pipet, the excess is removed by touching the tip of the pipet to filter paper. The test solution is then transferred to the spot paper by touching the tip of the capillary pipet to the center of a yellow spot produced by the chromate solution. The silver solution is slowly drawn into the paper, and a brownish red spot forms around the tip of the pipet. If the quantity of silver is very small, the brown spot may appear 2 to 5 minutes after the addition of the test solution. The test is made more distinct by adding with the capillary pipet 10 to 30 λ of distilled water to the center of the brown spot. The water spreading in the paper removes the soluble yellow chromate and leaves the colored spot in an otherwise white field. For the purpose of confirmation, the brown spot is finally treated with a drop of 1 per cent sodium chloride solution. The silver chromate is converted to silver chloride, and the brown spot disappears immediately.

Mercuric mercury, cadmium, tin, and antimony do not give a test. Lead and bismuth produce yellow spots. The brown spot obtained with cupric ion after rinsing with water remains unchanged when treated with sodium chloride.

### EXPERIMENT 13

#### Lead Iodide

L.I., 0.2 γ Pb  
Pb:Bi = 15:1

The test is carried out in the same manner as the test for mercury. If a small quantity of bismuth is simultaneously present, the lead iodide forms a yellow core in the otherwise brownish black spot pro-

duced by the bismuth iodide. A nearly quantitative separation from the bismuth must precede this confirmatory test because of the unfavorable limiting proportion. The yellow spot of lead iodide disappears on addition of stannite reagent.

### EXPERIMENT 14

#### Quinine Iodobismuthite <sup>12</sup>

L.I., 0.1 γ Bi

Drops of approximately 1-λ volume of bismuth, cupric, and antimony solutions containing 10 mg of metal ion per milliliter are transferred to spot paper by means of capillary pipets. Confusion will be avoided by writing the symbols of the metals with pencil on the paper so as to indicate the location of the drops. When the solutions have evaporated on the paper, small volumes of quinine-iodide reagent are added by means of a glass rod or dropper. The color of the spots is recorded, and, when the tests have dried, one of each kind is treated with a small drop of freshly prepared stannite reagent.

Only bismuth gives an orange-red quinine precipitate, which is reduced to black metallic bismuth on addition of stannite. The quinine iodoantimonite is orange-yellow in color and dissolves in stannite reagent to a colorless solution. The brownish black spot obtained with copper remains nearly unchanged when stannite is added. Mercuric mercury, cadmium, tin, and arsenic do not visibly react with the quinine-iodide reagent. Lead gives yellow  $PbI_2$ , which dissolves in the stannite reagent; the pale yellow  $AgI$  is reduced by stannite to black metallic silver.

### EXPERIMENT 15

#### Cupric Rubeanic <sup>13</sup>

L.I., 0.006 γ Cu

Approximately 1 λ of copper test solution (10 mg Cu per ml) is transferred to spot paper by means of a capillary pipet. The moist spot is exposed to fumes of ammonia by holding the paper over the opening of a bottle containing strong ammonia solution. Finally, a drop of a 1 per cent solution of rubeanic acid in alcohol is added. A bluish black spot is produced by the formation of cupric rubeanate.

<sup>12</sup> E. Léger, *Z. anal. Chem.*, **28**, 374 (1889); M. Cefola, Ph.D. Thesis, New York University, 1941.

<sup>13</sup> Prijadarajan Ray, *Z. anal. Chem.*, **79**, 94 (1929).

More dilute solutions of cupric salt produce a light, bluish gray spot with a dark core. The diameter of the dark core is related to the quantity of copper present. The test is specific for copper; only nickel gives a similar coloration.

### EXPERIMENT 16

#### Cadion 3B Lake of Cadmium Hydroxide <sup>14</sup>

L.I., 0.000 1 γ Cd.

Cd: (Cu, Bi, Sb, As, Al, Cr, Co, Ni, Mn, Be, Mg) = 1 : 5000.

Cd: (Pb, Zn, Na, K, NH<sub>4</sub>) = 1 : 10,000.

Cd: Ca = 1 : 1000.

Quantitative filter paper (Schleicher & Schüll, No. 589 <sup>3</sup>) or spot paper is soaked with 0.02 per cent alcoholic solution of Cadion 3B, benzenediazoaminobenzene-4-azo-4'-nitrobenzene, pressed between blotting paper or paper towels, and allowed to dry. The reagent paper, which has a yellow color, may be cut into strips approximately 15 mm wide and stored.

Drops of cadmium test solution (10 mg Cd per ml) and of Rochelle buffer solution are deposited on a clean glass plate, and 1-cm length of each solution is taken up in a capillary pipet. By blowing with the mouth, the contents of the pipet are transferred to a clean part of the glass plate and mixed with a glass thread. Approximately 1 λ of the mixture is taken up into a capillary pipet with a fine tip and transferred to the Cadion reagent paper. When the cadmium solution has evaporated (heating is not permitted), a drop of a mixture of 4 volumes of 2 M potassium hydroxide solution and 1 volume of ethyl alcohol is added with a glass rod. The reagent paper turns purple, and the circular area in which the cadmium was adsorbed becomes pink or salmon red. Addition of a drop of freshly prepared stannite reagent causes no appreciable change of color.

Mercuric ion gives a yellow spot within the purple stain, which might be mistaken for a cadmium test. When stannite is added, however, the yellow spot changes to gray. Tin solutions produce a white spot in the purple area. Other common elements of the hydrogen sulfide group do not produce a visible effect.

The diameter of the pink spot is related to the quantity of cadmium present. The reagent, Cadion 3B, is destroyed by heat or strongly acid solutions.

<sup>14</sup> F. P. Dwyer, *Australian Chem. Inst. J. & Proc.*, 4, 26 (1937); 5, 32 (1938).

## EXPERIMENT 17

Silver Arsenate<sup>15</sup>

L.I., 1 γ As

Drops of approximately 1-λ volume of slightly acid or alkaline arsenate solution containing 10 mg of arsenic per milliliter are transferred to quantitative filter paper (Schleicher & Schüll, No. 589<sup>s</sup>) by means of a capillary pipet. The paper is laid aside to dry, and then 5 to 10 λ of buffered silver nitrate solution (Appendix) is added to each test by means of a capillary pipet.

The appearance of a brown spot of  $\text{Ag}_3\text{AsO}_4$  proves the presence of arsenate ion. Antimony and stannic tin do not produce colored reaction products. A quantity of chloride ion equal to that of arsenate ion does not hinder the detection of arsenate, but the appearance of the brown spot is slightly delayed.

## EXPERIMENT 18

Cinchonine Iodoantimonite<sup>16</sup>

L.I., 0.5 γ Sb

Drops of approximately 1-λ volume of antimony, bismuth, and lead solutions containing 10 mg of metal ion per milliliter are transferred to spot paper by means of capillary pipets. When the solutions have evaporated on the paper, small drops of Korenman's cinchonine-iodide reagent are added by means of a glass rod or dropper. After the colors produced have been recorded, one test of each kind is immediately treated with a large drop of iodide-acetate reagent (Appendix).

The cinchonine iodobismuthite produces a vivid orange-red spot which is not much changed by the addition of the iodide-acetate reagent. The corresponding antimony compound gives an orange-yellow spot which turns immediately white when the iodide-acetate reagent is added. A brown ring may form around the white central portion but it disappears when the spot dries. The yellow lead iodide remains unchanged when the iodide-acetate reagent is added; a brown ring forming around the yellow core disappears when the paper dries. The pale yellow silver iodide cannot be mistaken for the antimony

<sup>15</sup> F. Feigl and J. W. Matthews, *Qualitative Analysis by Spot Tests*, New York, Nordemann Publishing Co., 1937.

<sup>16</sup> I. M. Korenman, *Z. anal. Chem.*, 99, 402 (1934).

compound. Cupric ion produces a blackish brown spot. Stannic ion gives a blue spot; mercuric mercury, cadmium, and arsenic do not yield colored products.

### EXPERIMENT 19

#### Molybdenum Blue Test for Tin<sup>17</sup>

L.I., 1 γ Sn  
Sn : Cu = 4 : 1

By means of scissors, magnesium ribbon is first cut into strips of approximately 1-mm width. Then a piece of black paper is covered with a clean glass plate, and small rectangular pieces, approximately 0.5 by 1 mm, are cut off the 1-mm strip so that the pieces drop onto the glass plate.

A drop of approximately 1-λ volume of stannic chloride test solution (10 mg Sn per ml) is taken up into a capillary pipet. A large drop of concentrated hydrochloric acid is deposited on a slide, and 1 λ of this acid is allowed to enter the pipet containing the tin solution. The contents of the pipet are transferred to a slide, and a piece of metallic magnesium is placed into the droplet of strongly acid tin solution. This is done best by first dipping the end of a glass thread in the drop of tin solution, and then touching the magnesium particle with it. The metal adheres and is easily transferred to the test drop.

When the evolution of hydrogen has stopped, the test drop, which may contain metallic particles, is taken back into the capillary pipet and immediately transferred to ammonium phosphomolybdate test paper (Appendix). A blue spot appears immediately, and it becomes more distinct when the paper is laid aside and allowed to dry.

The test is repeated with 1 λ of cupric test solution (10 mg Cu per ml). A blue spot is obtained, which disappears when the paper is laid aside to dry. The presence of copper promotes also the fading of the molybdenum blue produced by stannous ion, if there is not at least four times more tin present than copper.

Arsenic, antimony, and mercury solutions do not give reduction of ammonium molybdate to molybdenum blue under the conditions specified for the tin test.

### SLIDE TESTS

Typical slide tests are based upon the separation of more or less coarsely crystalline precipitates. The reactions are performed on

<sup>17</sup> F. Feigl and F. Neuber, *Z. anal. Chem.*, **62**, 382 (1923).

microscope slides, and the shape of the crystals of the precipitate is observed under the microscope.

When comparing the different techniques for the performance of confirmatory tests with regard to specificity and reliability, the slide tests must be given the highest rating. With spot tests and fiber tests the outcome rests essentially upon the observation of color phenomena. At times it will be impossible to tell whether or not a new phase has formed. Test tube tests are somewhat superior, for, aside from the color effects, the separation of precipitates and their general appearance can be observed without difficulty. In addition to these criteria slide tests reveal the shape of the particles of the precipitate. The convincing finality of microscopic identification recommends slide tests for general use in macroanalysis and microanalysis.

Historically, the development of slide tests by Harting,<sup>18</sup> Helwig,<sup>19</sup> Wormley,<sup>20</sup> Bořický,<sup>21</sup> Haushofer,<sup>22</sup> Streng,<sup>23</sup> Klement and Renard,<sup>24</sup> and Behrens<sup>25</sup> not only preceded the evolution of the microtechnique of qualitative analysis, but actually gave the impulse for this later development. Thus, it is easy to understand why slide tests and microanalysis, to many a mind, seem to merge into an inseparable whole. It is more appropriate, however, to assign the slide tests a separate position in the framework of qualitative analysis.

The distinctive feature of slide tests is determined by the use of crystallographic criteria rather than by the more or less incidental necessity of employing a microscope for observation. Since the particles of precipitates are frequently so small that even the microscope

<sup>18</sup> P. Harting, *Das Mikroskop; Theorie, Gebrauch, Geschichte und gegenwärtiger Zustand desselben*, translated into German by F. W. Theile, Braunschweig, Fr. Vieweg u. Sohn, 1859.

<sup>19</sup> A. Helwig, *Das Mikroskop in der Toxikologie*, Mainz, 1865.

<sup>20</sup> Th. G. Wormley, *Micro-Chemistry of Poisons*, etc., 2nd ed., Philadelphia, J. B. Lippincott Company, 1885.

<sup>21</sup> E. Bořický, "Elemente einer neuen chemisch-mikroskopischen Mineral- und Gesteinsanalyse," *Arch. naturw. Landesdurchforschung von Böhmen*, 3, Prague, 1877. Translated into English by N. H. Winchell, *19th Annual Report of the Geological and Natural History Survey of Minnesota*, Minneapolis, 1892.

<sup>22</sup> K. Haushofer, *Mikroskopische Reaktionen, Eine Anleitung*, etc., Braunschweig, Fr. Vieweg u. Sohn, 1885.

<sup>23</sup> A. Streng, *Neues Jahrb. Mineral. Geol.*, 1885, 21; *Ber.*, 18 (3) 84 (1885).

<sup>24</sup> C. Klement and A. Renard, *Réactions microchimiques à cristaux et leur application en analyse qualitative*, Brussels, A. Manceaux, 1886.

<sup>25</sup> H. Behrens, *A Manual of Microchemical Analysis*, with an introductory chapter by J. W. Judd, London and New York, Macmillan & Co., 1894. H. Behrens, *Anleitung zur mikrochemischen Analyse*, 2nd ed., Hamburg and Leipzig, Leopold Voss, 1899.

is unable to reveal their shape distinctly, the reactions employed for slide tests are intentionally selected and conducted so as to produce relatively large crystals, the dimensions of which range from a few microns to a few millimeters. Either the precipitates are rather soluble, or they are made to separate slowly by continuously maintaining a low degree of supersaturation. In some instances crystals of sufficient size are obtained by recrystallization from suitable solvents.

Examples of the various techniques employed in the performance of slide tests are given in Expts. 20 to 31. The reagent is frequently added in the solid state so that it spreads slowly by diffusion throughout the test drop. In some instances, the liquid reagent is added to the residue obtained on evaporation of the test drop, or test and reagent drops are placed side by side on the microscope slide, and are joined by connecting them by a narrow channel. It is always important to begin the microscopic examination at latest immediately after addition of the reagent. Observation of the dissolution of the reagent and of the subsequent crystallization of the test form demonstrates the presence of the sought-for substance in a very convincing manner. If the test is set aside after the addition of the reagent, and the crystalline precipitate is observed some time later, one cannot be absolutely certain whether the crystals have been formed by the action of the reagent or because of the evaporation of the solvent. To arrive at a decision, it will then be advisable to inspect another test drop which has been allowed to stand for an equal length of time without addition of reagent.

The time of appearance and the location of the precipitate in the test drop provide valuable clues which help to prevent misinterpretations. Crystals which are slowly growing along the edges of the drop form because of the increase in concentration by evaporation of the solvent. Crystallization caused by the immediate action of the reagent takes place close to the reagent, and, as a general rule, the closer the crystals are to the reagent the smaller is their size. The general appearance of tests makes it possible also to draw conclusions as to the conditions in the test drop, and the information may be used for improvements toward a more successful repetition of the tests. If the solid reagent does not dissolve or dissolves very slowly in the test drop and very few small crystals or none of them separate close to the reagent, the concentration of the sought-for substance probably is extremely high. The crystals of the solid reagent cannot dissolve, since they have become coated with the insoluble test form. If small crystals separate in large numbers close to the reagent, the concentration of the sought-for substance is very high; larger crystals

may form after some time at a distance from the reagent, but even these may be quite small. If small crystals separate close to the reagent and large ones form at a distance, the best conditions prevail; with still lower concentrations of the sought-for substance, the number of small crystals close to the reagent decreases, and there is a distinct trend toward the formation of medium-sized crystals only. With increasing dilution of the test solution, size and number of the crystals of the test form decrease. Finally, no precipitate separates immediately after addition of the reagent, and the crystals appear after some time and mostly along the edge of the drop where the effect of evaporation is most pronounced.

The volume of the drops used for slide tests is optional. Of course, the limits of identification improve in proportion with the reduction of volume. A limit is set, however, by the necessity of having sufficient matter in the test drop for the formation of crystals of a size sufficient for observation of the shape. As a rule, far more than  $0.005 \gamma$  of the sought-for ion is needed, which shows that slide tests cannot be used in the microanalysis of extremely small samples. In the following experiments, drops of  $0.3$ - to  $1\lambda$  volume are employed so as to render the tests directly applicable to the analysis of milligram samples. Much smaller drops are impracticable because of rapid evaporation.

## EXPERIMENT 20

### Mercuric-Cobaltous Thiocyanate<sup>26, 27, 28</sup>

L.I.,  $0.01 \gamma$

A drop of mercuric test solution (10 mg Hg per ml) and a drop of 2 *M* nitric acid are placed, side by side, on a microscope slide. Into a capillary pipet of approximately uniform bore is taken up a 5-mm column of the mercuric test solution; then, without emptying the pipet, its tip is inserted into the drop of nitric acid. The acid is allowed to rise in the pipet until the length of the entire liquid column has reached 50 mm. Now the contents of the pipet are transferred to a slide and mixed with a glass thread. A few portions of approximately  $1\lambda$  volume of the mixture are taken up with the capillary pipet, deposited near the end of a clean slide, and there evaporated just to dryness. Each residue will contain approximately  $1 \gamma$  of mer-

<sup>26</sup> P. T. Cleve, *J. prakt. Chem.*, **91**, 227 (1864).

<sup>27</sup> H. Behrens and P. D. C. Kley, *Mikrochemische Analyse*, Leipzig and Hamburg, Voss, 1915.

<sup>28</sup> N. Schoorl, *Z. anal. Chem.*, **47**, 209 (1908).

cury. After the slide has cooled to room temperature, one of the residues is treated with a very small volume of ammonium-cobaltous thiocyanate reagent.<sup>20</sup> The reagent is best added with a capillary pipet so that only part of the residue is covered by the reagent drop.

Inspection of the test under the microscope reveals clusters of deep blue, spear-shaped crystals of  $\text{CoHg}(\text{CNS})_4$ . The test may be repeated with the other residues. Breathing on the residues before adding the reagent may lead to the formation of larger crystals. Since the double salt has a tendency to form supersaturated solutions, it may sometimes happen that a deep blue test drop is obtained but no crystals separate. Seeding is performed with the use of a test drop in which satisfactory crystallization has been obtained. A small crystal of the precipitate is removed by means of a fine platinum wire or glass thread and transferred into the edge of the supersaturated drop. The edge of the drop is focused under the microscope, using a magnification of 20 to 30, and then it is touched with the "infected" needle while observing through the microscope.

On evaporation of the test drops, the excess of reagent crystallizes in light-blue aggregates which are easily distinguishable from the crystals of the precipitate.

### EXPERIMENT 21

#### Silver Dichromate<sup>22</sup>

L.I., 0.2 γ Ag

Approximately 5 λ of silver test solution (10 mg Ag per ml) is transferred to a microscope slide by means of a clean platinum loop. Every drop of solution is deposited by repeatedly touching the loop to the surface of the slide until the loop is empty. Each loopful gives several droplets which are deposited within as small an area as possible. With the same loop, 1 λ of 16 *M* nitric acid is deposited close to the silver solution, and all the small droplets are stirred together. If the nitric acid is taken from a large drop deposited on a slide, the loop may be used for transference without cleaning.

The acidified silver solution is now spread out over a large area. For this reason it is taken up in a capillary pipet and transferred to another slide. With transmitted light and a magnification of 30 to 80, the edge of the drop is focused under the microscope so as to be ready for microscopic observation immediately after addition of the reagent.

<sup>20</sup> B. Ormont, *ibid.*, 70, 308 (1927).

A glass thread for the transference of the solid reagent is freshly drawn out, as illustrated by Fig. 23, from a short piece of capillary of approximately 0.5-mm bore. A glass bead is fused in the center of the tube and drawn out, after removal from the flame, to a thread of about 0.2-mm diameter.

**ADDING SOLID REAGENTS.** A few grains of solid potassium dichromate are transferred from the reagent vial to a slide by means of a clean spatula. A kernel of 1-mm diameter (0.5-λ volume) is transferred to the test drop as follows. The point of the glass thread is moistened by dipping it into the test drop to which the reagent is to

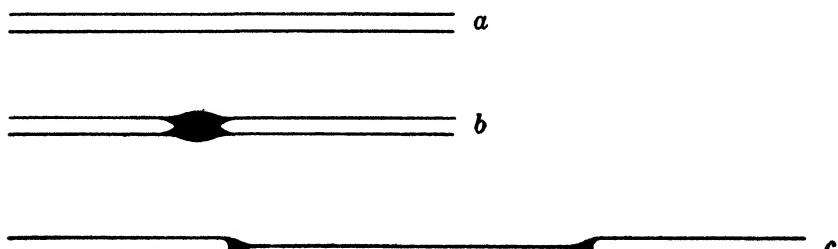


FIG. 23. Preparation of glass threads. The bore of the capillary is intentionally exaggerated.

be added. The selected grain is touched with the point of the thread, lifted up, and placed on the edge of the test drop. Glycerol, organic solvents, or distilled water might be used for moistening the point. The use of saliva is not advisable because of the inorganic ions contained in all body liquids. The removal of reagent directly from the vial with a moistened needle is objectionable for obvious reasons. It is also to be understood that unused portions of reagent must not be returned to the reagent bottle or vial, and that the glass thread is never used more than once; at least that portion of the thread which has come in contact with the reagent or solution must be broken off and discarded.

The test drop is observed under the microscope *immediately* after the addition of the reagent so as to actually *see* the solution of the reagent and the formation of a new solid phase. The crystals of  $\text{Ag}_2\text{Cr}_2\text{O}_7$  are yellow to reddish orange in color, depending on the thickness; they belong to the triclinic system and exhibit birefringency and a slight degree of pleochroism (light to dark). A sketch of the characteristic forms observed is an excellent aid to the memory.

**DRYING OUT OF TEST DROPS.** On evaporation of the test drop, all the solids in solution will crystallize. In the silver dichromate test,

crystallization of the potassium nitrate formed and of the excess of dichromate must be expected. The recognition of the characteristic forms in the crystalline mass left after evaporation is usually difficult and often impossible. Therefore, the rapid evaporation of test drops in hot, dry weather is annoying. Covering the test drop with a 1-inch watch glass or breathing on the slide from time to time will retard the evaporation.

### EXPERIMENT 22

#### Silver Chloride <sup>22</sup>

L.I., 0.1 γ Ag

Slow crystallization from ammoniacal solution produces microscopic crystals of silver chloride.

Solid silver chloride is transferred to a slide with a spatula. A particle of 0.5-mm diameter (0.05-λ volume) is pushed to an empty

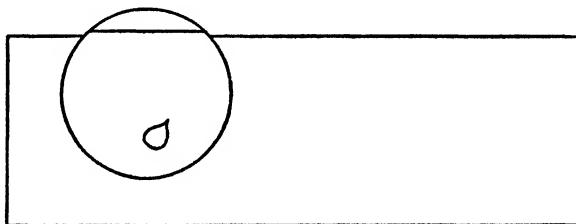


FIG. 24. Retarding evaporation.

portion of the surface of the slide and is dissolved there by the addition of 5 λ of 6 M ammonia solution. The ammonia is added with a calibrated capillary pipet; the mixture is stirred with a glass thread and immediately covered with a 1-inch watch glass so that it projects over the edge of the slide as shown in Fig. 24. The small opening between slide and watch glass permits slow escape of the ammonia, and the rate of its evaporation from the solution is sufficiently retarded so as to produce well-grown crystals of silver chloride.

The mixture is allowed to stand for 10 minutes. The separation of the silver chloride is indicated by the appearance of a turbidity which is visible with the unaided eye. The watch glass is finally removed, and the crystallization is examined under the microscope with a total magnification of 80 or more. If the test drop evaporates completely, a small piece of cover glass having a drop of 4 M nitric acid hanging on its lower surface is placed on the residue. The drop spreads between slide and cover slip and dissolves all the separated

solids but the silver chloride. Medium magnifications from 100 to 300 can be efficiently used after the addition of the cover glass.

The cubic forms observed in this preliminary experiment are not always obtained when the silver chloride has been separated from mixtures with other metallic ions. The presence of small quantities of lead appears to give rise to the formation of skeletal forms which separate as three-pointed stars. Traces of mercury seem to cause the formation of spherulites.<sup>30</sup> In spite of these interferences, the silver chloride is easily recognized by its extremely high refractive index, which renders the smaller crystals entirely black in transmitted light and gives the larger crystals heavy outlines. Observation with reflected light shows that the crystals are colorless.

### EXPERIMENT 23

#### Potassium-Copper-Lead Nitrite<sup>25</sup>

L.I., 0.003 γ Pb.

Pb : Cu = 1 : 300 and 1000 : 1.

Pb : Hg(ic) (as chloride) = 1 : 100.

"Large" quantities of cadmium or bismuth hinder the test.

**PREPARATION OF REAGENT.**<sup>31</sup> With a graduated pipet, 0.05 ml 5.8 *M* potassium nitrite solution and a like volume of acetate buffer solution (450 g sodium acetate, 100 ml glacial acetic acid, and water to make 1 liter solution) are transferred to a microcone and thoroughly mixed by stirring. The reagent, mixed ready for use, should be renewed every third day; it is perfectly safe to use it for 2 days, if the cone is stoppered with a cork and placed in a block which is not located in the neighborhood of strong sources of heat or light.

**PERFORMANCE OF THE TEST.** By means of a platinum loop, several small drops of lead test solution (10 mg Pb per ml) are deposited 5 to 10 mm apart near one end of a microscope slide. The slide is then held approximately 5 cm above a small Bunsen flame and the droplets are evaporated. To prevent overheating of the residues, the slide is heated only until evaporation starts; it is then removed and the evaporation is made complete by blowing on the drops with the mouth. The slide is quickly cooled to room temperature by laying it on a piece of metal, e.g., a metal block or the base of the microscope. By means of a capillary pipet, each residue is treated with a volume

<sup>30</sup> N. Schoorl, *Z. anal. Chem.*, **47**, 222 (1908).

<sup>31</sup> J. I. Adams, A. A. Benedetti-Pichler, and J. T. Bryant, *Mikrochemie*, **26**, 29 (1939).

of copper test solution (10 mg Cu per ml) which is approximately equal to the volume of the former drop of lead solution. Care must be taken not to spread the copper solution over areas larger than those covered by the residues of lead nitrate. Evaporation of the droplets is repeated in the manner described above, and the slide is again cooled to room temperature. Now, with a platinum loop or capillary pipet, a small volume of nitrite reagent is placed on one of the residues. The drop of reagent must be so small that it does *not* completely cover the residue. The test is immediately inspected under the microscope, using *strong* illumination with transmitted light and a total magnification of 70 to 100.

The small crystals of  $K_2PbCu(NO_2)_6$ , of 10- to 25- $\mu$  edge, form either immediately or, with small quantities of lead in a relatively large drop, within a few minutes. Their outlines are square or rectangular; the color varies from yellow to black, depending upon the thickness of the individual crystals. Bubbles of nitrogen oxides are readily recognized by their circular outline. When evaporation nears completion, various salts begin to separate and the recognition of the triple nitrite becomes difficult or impossible.

The appearance of the test clearly indicates the procedure to improve the test.

1. If no crystals of the triple nitrite are obtained, or only a few small ones grow near the edge of the drop within 5 minutes from the addition of the reagent, either the reagent is spent, or too much of the reagent has been taken; the triple nitrite is a rather soluble precipitate which necessitates keeping the volume of reagent at a minimum. The test is repeated by adding a very small volume of reagent to another residue. If no improvement is obtained, it is obvious that the reagent does not have the proper composition; a new batch is mixed. At times it becomes necessary to prepare a fresh solution of potassium nitrite.

It should be understood that the slow separation of a few small crystals of the triple nitrite must be expected whenever the quantity of lead approaches the limit of identification.

2. If a brown or black mass of precipitate forms immediately and the individual crystals are so small that their shape cannot be recognized, the degree of supersaturation in the test drop is too high and a great number of crystals separate so rapidly that no material is left for the slow growth of larger crystals. The test is repeated after dissolving the residues in a small amount of water obtained by breathing on the slide.

3. If large black crystals separate within 3 minutes, factors determining the formation of the precipitate are properly balanced.

The triple nitrite test is specific for lead, since no other element is known which could substitute for it without changing the appearance of the crystals. Barium, strontium, and calcium give precipitates consisting of crystals in shape similar to that of the triple nitrite of lead; the crystals of the barium compound, however, are colorless, and those produced with strontium or calcium are green in color. The place of the copper in the potassium-lead-copper nitrite can be taken by nickel without causing much change in the appearance of the crystals; rubidium, cesium, and thallous ions may substitute for the potassium and cause a decrease of the solubility of the precipitate. As to the quantity of copper to be added in the test for lead, the ratio  $Pb : Cu = 1 : 10$  gives the best results. It need not be strictly observed, however, since the limiting proportions admit large deviations.

#### EXPERIMENT 24

##### Cesium Iodobismuthite<sup>25, 32</sup> and Cesium Iodoantimonite<sup>25, 33</sup>

L.I., 0.01 γ Bi  
0.01 γ Sb

By means of a pipet, 5 λ of bismuth test solution (10 mg Bi per ml) is placed on a slide. The drop is evaporated just to dryness, and the residue is dissolved in 5 λ of 2 M nitric acid when the slide has again attained room temperature. A crystal of cesium chloride approximately 0.5 λ in volume (1 mm in diameter) is introduced into the drop near the edge. The reagent dissolves quickly, and colorless crystals of cesium chlorobismuthite separate around the point where the cesium chloride was introduced. Hexagonal plates are usually found in the midst of a variety of other forms.

Without removing the slide from the stage of the microscope, a crystal of potassium iodide, approximately 0.5 λ in volume, is introduced into the test drop near the edge and just outside the zone of precipitated cesium chlorobismuthite. Observation through the microscope will show yellow, red, and nearly black hexagons and stars forming where the zones of diffusion of the iodide and the cesium chloride meet. The intensity of color is, as usual, determined by the thickness of the crystals. When the diffusing iodide ion finally

<sup>32</sup> A. C. Huyssen, *Atlas zum Gebrauch bei der mikrochemischen Analyse*, 2nd ed., Leyden, E. J. Brill, 1932.

<sup>33</sup> N. Schoorl, *Z. anal. Chem.*, 47, 367 (1908).

reaches the colorless crystals of chlorobismuthite, they gradually become converted to orange cesium iodobismuthite. The preparation is set aside for later use, and the experiment is repeated using antimony test solution in the place of the bismuth test solution.

Since the chlorides of antimony are volatile, evaporation for the adjustment of the acidity must be omitted, and 5  $\lambda$  of the antimony trichloride solution (10 mg Sb per ml) is directly treated with the solid reagents. It will be noticed that antimony and bismuth react in practically the same manner. It may be mentioned, however, that solutions of tartar emetic,  $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$ , must be acidified with hydrochloric acid or they will not give the test.

The precipitation of the iodo complexes, probably  $Cs_3Bi_2I_9$  and  $Cs_3Sb_2I_9$ , is sufficient proof for the presence of either bismuth or antimony or both. Distinction between the two metals is possible by the addition of stannite reagent. The preparations are set aside for use in Expt. 26.

### EXPERIMENT 25

#### Bismuth Cobalticyanide <sup>24</sup>

L.I., 0.01  $\gamma$  Bi.

Bi : [Sb, As, Hg(ic), Ag, Cu(ic), Cd, Zn] = 1 : 1.

Bi : Pb = 1 : 12; Bi : Tl = 1 : 20; Bi : Sn(ous) = 1 : 5.

1 per cent chloride ion in solution prevents the test.

Approximately 5  $\lambda$  of bismuth test solution (10 mg Bi per ml) is transferred to a slide and evaporated just to dryness. The residue is dissolved in 5  $\lambda$  2 M nitric acid, and a crystal of potassium cobalticyanide of 0.5- $\lambda$  volume (1-mm diameter) is introduced into the edge of the drop of solution. With transmitted light and a total magnification of 70 to 100, the crystallization of  $BiCo(CN)_6 \cdot 5H_2O$  is observed under the microscope. Close to the reagent the precipitate appears as a fine powder, but larger crystals grow at the outermost edge of the zone of precipitation and exhibit very characteristic shapes. The crystals grow rather slowly, and it is advisable to continue the observation for about 5 minutes. Remarkable twin crystals are occasionally observed. The preparation is finally set aside for use in Expt. 26.

The form of the crystals depends in general to a large degree on the conditions under which the crystallization occurs. Often, the presence of foreign substances has a decisive influence on the microscopic appearance of tests. Among others, the crystals of bismuth

<sup>24</sup> A. A. Benedetti-Pichler, *Z. anal. Chem.*, **70**, 257 (1927).

cobaltcyanide are modified by the presence of lead, stannous, or mercuric ions. The startling change caused by the presence of a high concentration of lead can be easily demonstrated by repeating the cobaltcyanide test exactly as described above, but using bismuth-lead test solution containing 50 mg of lead and 5 mg of bismuth in a milliliter. The resulting precipitate consists of lens-shaped forms which, seen from the front, exhibit the appearance of oil drops. Side views possess sharp outlines. The change of the form of the crystals is obviously caused by the presence of lead (approximately 10 per cent of it) in the lattice. The preparation should be saved for the next experiment.

### EXPERIMENT 26

#### Pseudomorphs of Metallic Bismuth <sup>25</sup>

**PREPARATION OF STANNITE REAGENT.**<sup>24</sup> Stannite reagent is prepared by adding 3 to 4 drops of stannous chloride reagent to 6 ml of 2 M sodium hydroxide solution and mixing. A clear, colorless solution must result. Since, on standing, decomposition produces a precipitate of metallic tin, the stannite reagent must be mixed just before use. The alkaline reagent is especially *dangerous to optical glass*, and special care must be taken not to get any of it on the front lenses of the microscope.

**PERFORMANCE OF THE TEST.** The preparations obtained in Expts. 24 and 25 should be allowed to stand until the tests have evaporated to dryness. Then, using a total magnification of 30 to 50, illumination with reflected light, and a green or blue background, one preparation is focused after the other and treated with a relatively large drop of stannite reagent, which is best added by means of a wide capillary. All the bismuth precipitates turn black; many of the crystals retain their original outlines. The black forms consist of metallic bismuth and must be considered pseudomorphs; their shape is determined by their history but bears no relation to the internal structure. The antimony compounds dissolve in the alkaline stannite solution.

### EXPERIMENT 27

#### Copper Ferrocyanide <sup>22, 24, 25</sup>

L.I., 0.05 γ Cu

Copper test solution is diluted with 9 volumes of water and approximately 1 λ of the diluted solution (1 mg Cu per ml) is placed

<sup>25</sup> F. Emich, *Lehrbuch der Mikrochemie*, Munich, J. F. Bergmann, 1926, p. 153.

in the center of a microscope slide. The test drop is made strongly ammoniacal by inverting the slide and placing it for one minute on the opening of a bottle containing concentrated ammonia solution so that the drop is exposed to the fumes. Then, by means of a glass thread, a kernel of potassium ferrocyanide of 0.8-mm diameter (0.25- $\lambda$  volume) is added, and the drop is immediately covered with a small watch glass to retard the evaporation of the ammonia. After 1 to 3 minutes the watch glass is removed, and the test drop is inspected under the microscope.

The crystals of the precipitate appear as long rectangles, coffin lids, needles, radial clusters of needles, and tufts of needles. The color varies from yellow to brown and even black, depending upon the thickness of the specimens. Plates with re-entrant angles, indicating twin formation, are quite common. Near the limit of identification only small diamonds and small needles are obtained. On evaporation of the test drop, the reagent crystallizes in large colorless plates along the edge of the drop.

### EXPERIMENT 28

#### Cadmium-Thiourea Reineckate <sup>86</sup>

L.I., 0.002 γ Cd

Approximately 0.5  $\lambda$  of diluted cadmium test solution (1 mg Cd per ml) may serve for the experiment.

The reagent must be freshly prepared. In a test tube, approximately 0.5 ml of water is treated with a few milligrams of Reinecke's salt (potassium tetrathiocyanodiamminochromate). The mixture is shaken, and after one minute 0.1 ml of the saturated solution is taken up into a pipet and transferred to another tube where it is mixed with 0.1 ml 2 *M* hydrochloric acid and 0.1 ml 5 per cent solution of thiourea. The reagent, which will keep for a few hours, is now ready for use.

The cadmium solution is transferred to a microscope slide and evaporated to dryness by alternately heating over a small Bunsen flame and blowing on the slide (mouth). The slide is then allowed to cool to room temperature, and the residue is dissolved in 1  $\lambda$  of 2 *M* hydrochloric acid. A large drop of the reagent solution (10 to 30  $\lambda$ ) is now added directly to the test drop by means of a glass rod or a capillary tube.

<sup>86</sup> C. Mahr, *Mikrochim. Acta*, 3, 300 (1938); C. Mahr and Hertha Ohle, *Z. anal. Chem.*, 109, 1 (1937).

Colorless crystals of  $\text{Cd}(\text{thiourea})_2[\text{Cr}(\text{CNS})_4(\text{NH}_3)_2]$  separate as characteristic X-shapes, rods, needles, and thick clusters of needles.

When testing for very small quantities of cadmium, it is preferable to add a small volume of reagent solution directly to the residue obtained on evaporation. It is furthermore advisable to retard the evaporation of the test by keeping the drop covered with a 1-inch watch glass. Near the limit of identification only a few tiny particles of precipitate are obtained, which slowly grow to small diamond shapes. To relieve supersaturation, it is advisable to scratch with the sharp edge of a broken-off glass thread.

Zinc and nickel do not give precipitates. Lead and bismuth give crystals which somewhat resemble those obtained with cadmium. In case of doubt, comparison with the precipitate obtained from a known cadmium solution will facilitate arriving at a decision. In addition, it should be noted that the precipitates of bismuth and lead as well as the precipitate obtained with copper solution have a light yellow or brown color. On evaporation the reagent itself leaves a residue of variously shaped needles.

### EXPERIMENT 29

#### Magnesium-Ammonium Arsenate<sup>25, 37</sup>

L.I., 0.05 γ As

With a capillary pipet are taken up from a platinum loop of approximately 1-λ capacity first four loops full of 2 M nitric acid and then one loop full of arsenate test solution (10 mg As per ml). The contents of the pipet are transferred to the center of a microscope slide. The slide is inverted and placed on the opening of a bottle containing strong ammonia so that the hanging test drop is exposed to the ammonia gas. It requires only a few minutes to render the test drop ammoniacal. The slide is then removed from the bottle, and a crystal of magnesium acetate of 0.1-λ volume (0.6-mm diameter) is added near the edge of the test drop. Observation under the microscope using a total magnification of approximately 80 and not too strong an illumination with transmitted light shows the colorless crystals of  $\text{MgNH}_4\text{AsO}_4 \cdot 6\text{H}_2\text{O}$ . The appearance of the crystals varies considerably, depending on the conditions during their formation. Clusters of feathery dendrites separate if the precipitation occurs instantaneously, while prismatic forms are the result of slow

<sup>37</sup> N. Schoorl, *Z. anal. Chem.*, 47, 367 (1908).

crystallization. Characteristic X-shaped forms are nearly always found, no matter which of the above-mentioned two types prevails otherwise. The magnesium-ammonium phosphate behaves in exactly the same manner, and it is advisable to convert magnesium-ammonium double salts into the corresponding silver salts for the confirmation of arsenate and phosphate, respectively. The preparation is set aside for complete evaporation and saved for the next experiment.

### EXPERIMENT 30

#### Silver Arsenate<sup>38</sup>

When the preparation obtained in the foregoing experiment has dried out completely, a large drop of 1 *M* ammonia is placed on the residue so as to cover it completely. After half a minute the wash liquid is drawn off by using a technique which was introduced into microanalysis by H. Behrens.<sup>39</sup>

**DRAWING OFF A SOLUTION FROM A PRECIPITATE.** Filter paper is cut into squares of 2-cm edge. A corner of such a square is inserted into the edge of the drop, Fig. 27a, and the slide is inclined so that the liquid must flow upward to reach the paper. This procedure retards the flow and decreases the loss of precipitate. Depending upon whether the precipitate adheres firmly to the glass or not, the removal of liquid may be speeded up by holding the slide horizontally or even inclined toward the paper. Complete removal of the liquid is impossible without disturbing the precipitate. Thus, when most of the wash liquid has been taken up by the paper, the slide is placed on a horizontal support, and another large drop of 1 *M* ammonia is added. Less material will be lost if the drop is not placed directly on the precipitate but deposited near the area occupied by the precipitate so that it slowly spreads and finally covers the precipitate. Of course, the flow of wash liquid may be regulated by properly inclining the slide and, if necessary, assisting with the sealed end of a capillary. The second drop of wash liquid is removed in the same manner as the first, and the washing is repeated twice more. It is obvious that the washing will be most efficient if addition and removal of liquid are performed at points opposite to each other at the circumference of the original test drop. In this way successive portions of wash liquid will flow across the area occupied by the precipitate. Microscopic inspection of the precipitate after each washing is advisable.

<sup>38</sup> F. Emich, *Mikrochemisches Praktikum*, Munich, J. F. Bergmann, 1924, p. 90.

<sup>39</sup> H. Behrens, *Anleitung zur mikrochemischen Analyse*, 2nd ed., Hamburg and Leipzig, Leopold Voss, 1899, p. 21.

After washing, the slide is set aside until it has become completely dry. Then characteristic portions of the precipitate are focused with the microscope, using a suitable magnification, illumination with reflected light, and a blue or green background. With a platinum loop, neutral 1 per cent silver nitrate solution (silver test solution) is added to individual crystals of magnesium-ammonium arsenate while observing through the microscope. The color of the crystals changes to reddish brown, but the outlines are retained as a rule, since the reaction produces pseudomorphs of  $\text{Ag}_3\text{AsO}_4$ .

If so desired, Expts. 29 and 30 may be repeated with phosphate test solution substituted for the arsenate solution.

### EXPERIMENT 31

#### Rubidium Chlorostannate<sup>22, 40</sup>

L.I., 0.01 γ Sn  
 $\text{Sn : Sb} = 1 : 100$

With a platinum loop approximately  $1 \lambda$  of stannic test solution (10 mg Sn per ml) is transferred to a capillary pipet. A large drop of 3 M hydrochloric acid is placed on a slide, and, with the uncleared platinum loop, 5 loop fillings of the acid are transferred to the stannic solution in the pipet. The contents of the pipet are transferred to a microscope slide, and a crystal of rubidium chloride of  $0.1\lambda$  volume (0.6-mm diameter) is placed in the edge of the drop. Inspection under the microscope shows a fine powder where the reagent has been introduced and isometric crystals of  $\text{Rb}_2\text{SnCl}_6$  outside the zone of rapid precipitation. Octahedra and tetrahedra predominate.

The same type of crystal is obtained if cesium chloride is substituted for the rubidium salt. The crystals are smaller, however, because of the lower solubility of  $\text{Cs}_2\text{SnCl}_6$  and the consequently more rapid crystallization. The use of rubidium chloride seems preferable for the confirmation of tin, since cesium has a greater tendency to form insoluble compounds and is, therefore, more inclined to give like precipitates with other ions.

### FIBER TESTS

The use of single textile fibers as carriers of precipitates and colorations has been introduced by Emich.<sup>41, 42</sup> The test solution is evaporated on the end of a fiber which is treated with reagent either be-

<sup>40</sup> N. Schoorl, *Z. anal. Chem.*, **47**, 367 (1908).

<sup>41</sup> F. Emich, *Monatsh.*, **22**, 670 (1901); **23**, 76 (1902).

<sup>42</sup> F. Emich, *Ann.*, **351**, 426 (1907).

forehand or afterwards. Limits of identification ranging from 0.0002 to 0.01 γ may be obtained if the reaction product is intensely colored. The similarities between fiber tests and spot tests are obvious, but one must not forget that individual fibers lack the porosity and adsorptive power of paper, the medium of spot tests. Thus it may happen that precipitates which give excellent spot tests on paper are not suited for the fiber technique, and vice versa. Furthermore, the capillary forces in paper seem to favor the separation of precipitates in a state of fine division, and it is known that for some substances the coloring power increases with a decrease of the particle size, whereas for other substances the phenomenon is exactly reversed.<sup>43</sup>

### EXPERIMENT 32

#### Turmeric Test for Boric Acid<sup>42</sup>

L.I., 0.0005 γ BO<sub>3</sub>.

BO<sub>3</sub> : (PO<sub>4</sub>, SiO<sub>3</sub>, Ca, Mg) = 1 : 100.

BO<sub>3</sub> : Fe(ic) = 1 : 10.

Ti, Zr, Hf, Mo, Cb, and Ta give similar colorations.

**SELECTING A FIBER AND FIXING IT TO THE POINT OF A GLASS THREAD.** The turmeric linen is handled with forceps and needles only. The fibers are cut into pieces of approximately 1-cm length, and five to ten of them are collected on a microscope slide. The fibers are inspected under the microscope in strong transmitted light, and an individual fiber is selected which exhibits a distinct yellow color over its entire length. In addition, the fiber should be as straight as possible, and the end to be used in the test must not be pointed. Now, a short capillary is drawn out to a fine glass thread, which is broken off approximately 2 cm from the capillary. The end of the thread is moistened with Canada balsam or some other kind of suitable cement, and then touched to one end of the selected fiber. The capillary with thread and attached fiber is then fastened to the body tube of a microscope, Fig. 25, with Plasticine or beeswax so that the free end of the fiber is approximately 1 cm above the stage of the microscope.

One platinum loop full of borate test solution (1 mg BO<sub>3</sub> per ml) is mixed on a slide with an equal volume of 6 M hydrochloric acid. A small fraction of the mixture, 0.1 to 0.5 λ, is transferred to the center of another slide by means of a capillary pipet. This slide is

<sup>43</sup> One may compare articles of C. P. van Hoek-Hilversum, *Farben-Ztg.*, 31, 1456 (1926), J. Parrish, *J. Oil Colour Chem. Assoc.*, 9, 255 (1926), and E. B. Spear and H. A. Endres, *Ind. Eng. Chem.*, 15, 725 (1923).

immediately placed on the stage of the microscope, the body tube is lowered, and the position of the slide is simultaneously adjusted so that the end, and only the end, of the turmeric fiber dips into the droplet as shown in Fig. 25. Continuous observation with the aid of a magnifying glass is advisable. The end of the fiber is held in contact with the liquid until the liquid has completely evaporated. Then

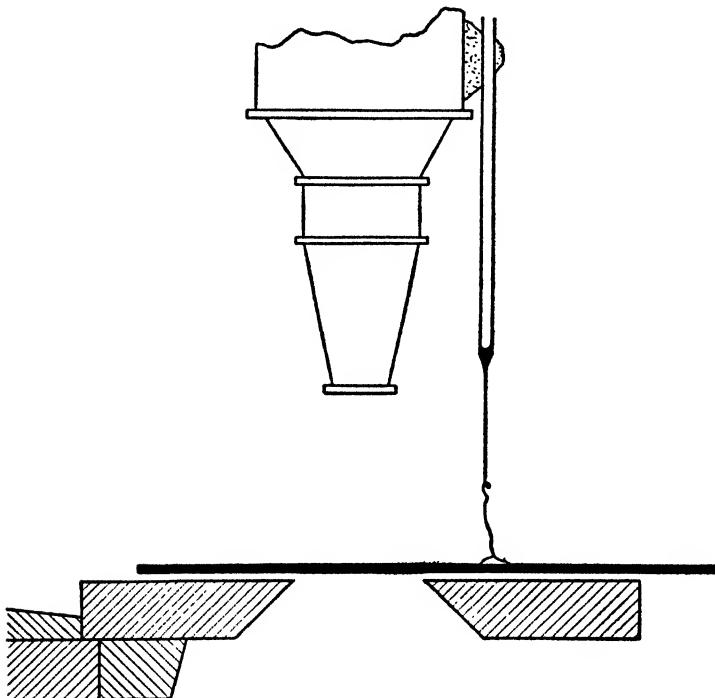


FIG. 25. Concentrating solutes on the point of the fiber. The thickness of the fiber is greatly exaggerated.

the body tube of the microscope is raised, and the capillary with the fiber is removed and placed on a clean slide.

**OBSERVATION OF FIBER TESTS.** The capillary may be attached to the slide with Plasticine as shown in Fig. 26. A dry cover slip is placed over the fiber to hold it close to the surface of the slide. The microscope is focused on the point of the glass thread. When the beginning of the fiber has been found, the slide is moved so that the entire length of the fiber passes through the field of vision. The free end of the fiber should now exhibit a brown or reddish brown color. The larger portion of the fiber should still possess the original yellow color.

If a microscope with polarizer in position is used, it is necessary to rotate the stage and to inspect the fiber in various positions. On a linen fiber, the red coloration exhibits very strong pleochroism from red to nearly colorless, and the test will be missed if the fiber is continuously kept in the "colorless" position.<sup>44</sup>

**TREATING THE FIBER WITH REAGENTS.** To further confirm the presence of borate, the free end of the fiber is focused with the microscope, and, by means of a glass rod or dropper, a large drop of 1 *M* ammonia is placed on the slide so that it touches the edge of the cover

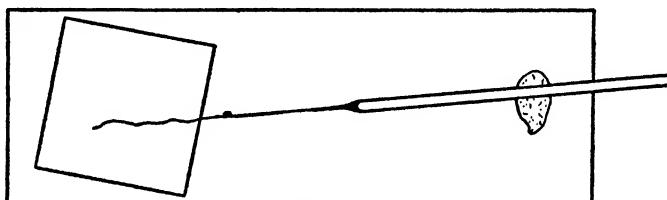


FIG. 26. Mounting fibers for examination. The thickness of the fiber is greatly exaggerated.

slip. The ammonia solution spreads between slide and cover glass, and the fiber becomes immersed. The color of the end of the fiber now changes to blue or green, while the yellow color of the rest of the fiber changes to red. The blue or green coloration of the tip fades quickly, which makes it advisable to observe immediately after adding the ammonia. Pleochroism (blue to colorless) is exhibited also by the blue portion of the fiber.

### EXPERIMENT 33

#### Bismuth Sulfide on the Fiber, Its Conversion to Sulfate, Chromate, and Elementary Bismuth

L.I., 0.008 γ Bi

A single fiber, the longest and straightest that can be found, is pulled out of a tuft of cotton by means of forceps. With scissors, the fiber is cut to a length of approximately 1 cm. A few drops of acetone are poured on a watch glass, and the fiber is bathed in the acetone so that all fat is removed from it. The fiber is then placed on a clean slide, picked up with a glass thread, and attached to the body tube of a microscope as outlined in the preceding experiment.

<sup>44</sup> F. Steidler, *Mikrochemie*, 2, 138 (1924).

A small drop of 0.1 to 1  $\lambda$  of bismuth test solution (10 mg Bi per ml) is placed on the center of a slide. The slide is transferred to the stage of the microscope, and the free end of the fiber is inserted into the drop. By repeated inspection during the evaporation of the drop one makes certain that not more than the very end of the fiber is in continuous contact with the drop.

**PRECIPITATION ON FIBERS.** After the drop has completely evaporated, the body tube of the microscope is raised, and a slide with a large drop of 1 per cent sodium sulfide solution is substituted for the one which carried the bismuth solution. The end of the fiber is dipped into the sulfide solution by lowering the body tube.

**RINSING THE FIBER AND MICROSCOPIC EXAMINATION.** After a few seconds, the body tube is slowly raised. The glass thread with the fiber is removed and placed on a clean slide, Fig. 26. A large drop of water is placed on a cover slip, which is then inverted and placed on the fiber. The fiber is examined under the microscope. Using reflected light and a white background, one is able to observe the black bismuth sulfide at the end.

**OXIDATION OF THE SULFIDE AND CONVERSION TO CHROMATE.** The glass thread is grasped and the fiber is carefully pulled out from under the cover slip. To facilitate handling, the fiber is left attached to the thread for the rest of the experiment. The fiber is inserted into the gas space of a bromine water bottle and held there for approximately 3 minutes. Care must be exercised not to touch the neck of the bottle with the fiber when it is inserted or withdrawn. The fiber would cling to the glass surface and be lost. Fiber and thread are then placed on a clean, *dry* slide, and the thread is covered with a *dry* cover slip. Microscopic inspection of the fiber with the use of reflected light and a colored background will demonstrate that the end of the fiber is white or colorless. The sulfide has been converted to sulfate.

Approximately 0.05 ml of 0.1 *M* potassium dichromate is placed at the edge of the cover slip so that the liquid is drawn under the glass by capillary attraction. When the fiber has become immersed in the solution, it is withdrawn by means of the glass thread and transferred to a 0.05-ml drop of water held ready on another slide. A cover slip is placed on the fiber, which is then inspected under the microscope by reflected light. The yellow  $(\text{BiO})_2\text{Cr}_2\text{O}_7$  is very difficult to recognize. A white background and a black background are worth trying.

**REDUCTION TO METALLIC BISMUTH.** The end of the fiber is focused under the microscope, and reflected light and a white background are

employed. Stannite solution is freshly prepared as outlined in Expt. 26. A large drop of the reagent is placed at one edge of the cover slip, and water is sucked off at the opposite edge by touching with a piece of filter paper. When the stannite solution reaches the fiber, the formation of the black precipitate of metallic bismuth is observed.

### TEST-TUBE TESTS

The procedure of test-tube tests is exceedingly simple. The test solution is treated with a reagent solution, and the mixture is examined for the appearance of a new phase (usually precipitate, turbidity, or gas) or a coloration. No attempt is made to determine the exact shape of the particles of a precipitate.

Tests based upon this simple principle are generally useful. The technique of performance and observation, of course, depends upon the volume of test solution available. If a large drop, 30 to 50  $\lambda$ , is available, the test may be performed in a centrifuge cone, in a microcone, or on a drop test plate. Microcones offer the advantage that small quantities of precipitates may be convincingly observed after being collected in the point of the cone by the application of centrifugal force, Expt. 48. The observation of weak colorations, however, is more sensitive in narrow capillaries, Expt. 46. If only a few lambdas of test solution can be taken, precipitations, too, are better carried out in capillaries, Expt. 44, which are drawn out to a fine point. For volumes of 5 to 500  $\text{m}\lambda$  of test solution, tiny centrifuge cones, the capillary cones, may be used, Expt. 57. An alternative for work with such small volumes is the performance of tests on the circular surface obtained by cutting a thin glass thread, Expt. 56.

It is obvious that the need for magnifying glass or microscope increases with the reduction of the volume of test solution. In working with capillary cones and in performing drop tests on the thread condenser, the low-power microscope is indispensable for the control of the manipulations. With magnifications of 400 and dark-field illumination for the observation of precipitates, tests may be performed on  $10^{-12}$  to  $10^{-13}$  liter (1  $\lambda\lambda$  to 0.1  $\lambda\lambda$ ) of solution. Aside from an elaborate microscope, micromanipulators and micro injection apparatus must be employed. The drop of test solution is either kept floating in a film of paraffin oil or it is deposited on the underside of a cover glass which forms the top of a moist chamber. The cover glass must be coated with a film of a suitable substance which repels the test solution and the reagent, and prevents their spreading. In this way the ultimate limits of identification for the following test-tube tests

were observed: 0.01  $\gamma\gamma$  Ba as barium sulfate,<sup>44</sup> 0.02  $\gamma\gamma$  Ag as silver chloride,<sup>45</sup> 0.2  $\gamma\gamma$  Co as cobalt-mercuric thiocyanate,<sup>45</sup> 0.4  $\gamma\gamma$  Fe as Prussian blue,<sup>4</sup> and 0.5  $\gamma\gamma$  Fe as ferric thiocyanate.<sup>45</sup> In all instances the limits were determined by the difficulties in the observation of the outcome of the tests. The manipulative technique permitted working with somewhat smaller drops than were employed in these experiments.

### BEAD TESTS

The limit of identification of bead tests may be improved by decreasing the size of the bead. The color of beads of 0.3-mm diameter can be easily observed, if they are fused on a straight platinum wire of 0.05- to 0.1-mm diameter.<sup>46</sup> The observation of the color is facilitated by immersing the bead in a liquid which has approximately the same refractive index as the bead.

### EXPERIMENT 34

#### Bead Test for Cobalt

L.I., 0.03  $\gamma$  Co

One end of a straight platinum wire of 0.05- to 0.1-mm diameter and approximately 2.5-cm length is fused into a wide glass capillary of 4-cm length, which serves as a handle.

Some borax is spread on a microscope slide, and a kernel of approximately 0.5- $\lambda$  volume (1-mm diameter) is selected for the experiment. By means of a platinum loop or a capillary pipet, the kernel is treated with approximately 0.2  $\lambda$  of diluted cobalt test solution (1 mg Co per ml). The wire is then heated to incandescence in the edge of a non-luminous Bunsen flame, and the kernel of borax is taken up by touching it with the end of the hot wire. The Bunsen flame is adjusted to a height of approximately 2 cm, and the bead is fused by heating the center portion of the wire in the edge of the flame about 5 mm above the orifice of the barrel. The bead tends to move toward the cooler parts of the wire. Thus, the bead may be kept at the point of the wire or driven back and forth over the length of the wire by proper application of heat. When the borax has been fused to a clear glass, the bead is allowed to cool. A large drop of xylene or water is placed near one end of a microscope slide, and the wire and

<sup>45</sup> Anne G. Loscalzo, unpublished experiments.

<sup>46</sup> F. Emich, *Mikrochemisches Praktikum*, Munich, J. F. Bergmann, 1924, p. 100.

handle are placed on the slide so that the bead is immersed in the liquid. Holding the slide over a brightly illuminated sheet of white paper, one will be able to observe the blue coloration with the aid of a magnifying glass. A microscope with a total magnification of 20 to 40 is preferable. The microscope is focused on the wire, whereupon the slide is moved to bring the bead into the field of vision. A condenser is desirable for concentrating the light on the bead. If the bead should be too small, more of the borax may be taken up by touching the kernel with the hot bead.

To clean the wire, the bead is fused again and the molten bead is thrown off the wire by a slinging motion with the hand. A relatively large amount of borax is then taken up and fused to a bead which is once driven back and forth over the wire and then thrown off. Repetition of this treatment will remove practically all the cobalt. The borax glass may be completely removed by finally placing the wire in dilute acid for 30 minutes.

#### DETERMINATION OF THE SENSITIVITY

The decision concerning a standard procedure for the performance of the test is a prerequisite for the determination of the sensitivity. The standard procedure must fix all factors which may affect the result of the test, as quantity of test substance, amount of reagent, method of bringing the two together, treatment of the mixture, means of overcoming supersaturation, time of waiting before observation, method of observation, and definition of a positive test. It is obvious that in the determination of the sensitivity one must rigidly adhere to the specifications of the standard procedure.

The limiting concentration and the limit of identification are determined simultaneously by applying the test to samples of standard size which contain decreasing amounts of the sought-for substance. Finally, the test is repeated several times with the mixture of greatest dilution which gave a positive result in the first series of trials. If all these final tests indicate the presence of the sought-for substance, the content of the mixture used may be taken as the limiting concentration and limit of identification.

The determination of the limiting proportions is carried out in an analogous manner. At first mixtures are prepared so that the concentration of the sought-for substance is approximately ten times higher than the limiting concentration. In the final experiments the concentration of the sought-for substance may be made equal to the limiting concentration. The concentration of the interfering substance

is gradually increased until the test becomes negative. After the experiment has been repeated several times with the most unfavorable ratio which gave a positive test, this ratio is taken as limiting proportion, if all trials give a positive result.

### EXPERIMENT 35

#### Silver Chromate and Silver Dichromate, L.C. and L.I. of Silver Test

10-ml graduate cylinder.

Measuring pipet, 2-ml.

12 test tubes, 6-in.

Test-tube rack.

6 microcones.

Spot paper.

Microscope.

0.5 ml of silver test solution diluted to 5 ml.

50 ml 0.01 *M* nitric acid, free from chloride.

10 ml 2 per cent ammonium chromate, free from chloride.

Five milliliters of the 0.1 per cent silver solution (1 mg Ag per ml) is diluted with an equal volume of 0.01 *M* nitric acid in a clean 10-ml graduate cylinder. Mixing is performed by pouring into a clean test tube, returning to the graduate cylinder, and again pouring into the test tube. Finally, 5 ml is returned to the graduate cylinder and again diluted with an equal volume of the acid so as to obtain the next higher dilution, and so forth. The test tubes with the solutions of increasing dilution are arranged in an orderly manner in the rack. Extreme care is required to prevent introduction of chloride ion, which would seriously alter the silver-ion concentration of the solutions. Four to five dilutions should be prepared altogether.

**SPOT TEST.** The procedure described in Expt. 12 is followed, the test being first tried with the solution of lowest silver-ion concentration and repeated with solutions of increasing concentration. It is best first to mark the concentrations with pencil on the spot paper. The drops of increasing concentration are then placed on the paper in rapid succession, and it is not necessary to clean the capillary pipet when proceeding from one concentration to the next higher. The test with the most dilute solution which gives a positive result is repeated ten times after the capillary pipet has been cleaned carefully. If all trials give positive results, the limiting concentration has been established, and the limit of identification is calculated. Otherwise the experiment is repeated with the solution of next higher concentration.

**TEST-TUBE TEST.** Beginning with the most dilute solution, 1 ml of the solution is taken up into a clean pipet. The remainder of the solution is poured out of the test tube and rejected, and the 1-ml portion is returned to the test tube. Without cleaning the pipet when proceeding to the solution of next higher concentration, the process is repeated until each test tube contains 1 ml of test solution. Using a clean pipet, each test solution is treated with 0.5 ml of 2 per cent ammonium chromate. After mixing (one must not close the opening of the tube with the thumb and shake), the tests are allowed to stand for 10 minutes, and then inspected for separation of a reddish brown precipitate. Repetition of the experiment with the lowest concentration which gave a precipitate may be omitted to save time.

**SLIDE TEST AND PREPARATION OF DILUTIONS ON A MICRO SCALE.** A capillary of approximately uniform bore of 0.5 to 1 mm and about 20 cm long is convenient to use. Clean slides for the performance of tests are held ready. A large drop of silver test solution (10 mg Ag per ml) is deposited on a slide, and a few milliliters of 2 *M* nitric acid are held ready in a watch glass. To begin with, the capillary is dipped into the acid, and the acid is allowed to rise to a height of 2 cm in the capillary. Then, after an equal length of the silver test solution has been taken up, the contents of the capillary are blown out into a clean microcone. Again 2 cm of nitric acid is taken into the capillary, and then 2 cm of the mixture in the microcone. Not more than six dilutions need be prepared.

Starting with the solution of lowest silver-ion concentration, 0.5  $\lambda$  of each dilution is transferred to a slide by means of a calibrated capillary pipet and immediately tested by adding a kernel of potassium dichromate of 0.05- $\lambda$  volume (0.5-mm diameter) as described in Expt. 21. All the tests may be easily performed on one slide. If no precipitate forms immediately after addition of the reagent, the drop is allowed to evaporate to dryness and the residue is examined for crystals of silver dichromate. If crystals of silver dichromate can be identified by their color and shape in either the drop or the residue of the drop, the test is considered positive. Finally, taking a fresh slide, the test with the critical dilution is repeated 10 times and, if all results are positive, the limit of identification is calculated from the determined limiting concentration and the volume of the test drop.

At the conclusion of the experiments the collected data should be assembled in table form, using gamma as the dimension for the limits of identification and milligrams of silver per milliliter as the dimension for the limiting concentrations. An interpretation of the findings should be attempted.

**LIMITING PROPORTIONS Ag : Pb.** A determination of the limiting proportion for the spot test and the slide test in the presence of lead may be tried. The silver-lead ratios 2 : 1, 1 : 1, 1 : 2, 1 : 5, and 1 : 10 are suggested for a trial. The quantity of silver ion in each test should be equal to ten times the limit of identification found in the preceding experiments.

## B. MICROTECHNIQUE OF QUALITATIVE ANALYSIS

Insufficient selectivity and unfavorable limiting proportions of most chemical confirmatory tests usually make it necessary to separate the components of complex mixtures before they are identified. Actual separation requires that parts of the mixture are transferred to different locations, and it is obvious that such a mechanical operation is possible only if these parts constitute different phases. Therefore, if the substances to be separated are originally in the same phase, they must be brought into different phases by chemical means so as to create the conditions required for mechanical separation. Frequently, the substances are in different phases but so intermixed as to render mechanical separation difficult. In such instances, too, chemical reactions are employed to bring the substances into easily separable phases.

The limitations of the so-called chemical separations are derived from two essentially different sources, from the *chemical reaction* producing the separable phases and from the *mechanical procedure* employed in their actual separation. The efficacy of the chemical reactions for qualitative work is described by their limiting proportions, which, within reasonable limits, are not affected by the quantity of reacting matter, as has been stated in a previous section. In contrast, the suitability of mechanical procedures depends greatly upon the scale on which the work is carried out. Thus, when transposing tested procedures to a different scale, attention must be focused upon the selection or development of proper manipulative technique and apparatus to permit the retention of the concentrations of the original method.

### MECHANICAL MANIPULATION UNDER THE MICROSCOPE

Microanalysis is intended for the investigation of small heterogeneities rather than for the determination of average composition. The object of analysis often is a small particle surrounded by extraneous matter. In such instances, the most obviously convincing and most conclusive procedure is the complete mechanical separation of the

specimen from the foreign matter before chemical analysis. This principle was clearly understood by François Vincent Raspail, and was practically applied by him as early as 1825 in his investigations on the formation of starch in the organs of plants.<sup>1</sup>

The special apparatus and technique depend essentially upon the size of the specimen and the amount of force required to separate it from its surroundings. Complications may arise if the specimen either is poorly defined or requires special methods of observation to render it visible.

A technique for dissecting metallic objects of various sizes under the microscope has been developed by Chamot<sup>2</sup> for the investigation of small arms ammunition. His tools and technique could be used for the removal of slags or corrosion products from metals. Clarke and Hermance<sup>3</sup> use the manipulator of Reichert (Vienna) for work of this type. The model was selected for its ruggedness of construction, and it was mounted on a sliding base so as to be continuously ready for use in conjunction with a petrographic microscope. As an alternative, a dental motor with flexible shaft and a set of drills, burrs, stones, and cutting wheels is used to reduce complex structures systematically under the microscope. Samples for analysis may be collected by covering the specimen with a drop of oil large enough so that the tiny burr employed is completely immersed. The particles which are removed by the burr are retained by the oil, and are subsequently recovered by centrifuging and washing with a suitable solvent. A binocular instrument serves for microscopic observation during such work. The simple microchemical manipulator of Alber<sup>4</sup> permits work with magnifications up to 200, and the application of considerable force is possible, since the tools are directly operated by hand. The mechanical manipulator, Expt. 56, with three rack-and-pinion motions is designed for approximately the same range of magnification but is less suited for the dissection of hard materials.<sup>5</sup> Micromanipulators, which permit the performance of mechanical operations on specimens requiring microscope magnifications of 1000 and more, are widely used in biological studies. Such instruments have

<sup>1</sup> E. M. Chamot and C. W. Mason, *J. Chem. Education*, **5**, 258 (1928); H. Harms, *Apoth. Z.*, No. 90 (1931).

<sup>2</sup> E. M. Chamot, *The Microscopy of Small Arms Primers*, New York, 1922.

<sup>3</sup> B. L. Clarke and H. W. Hermance, *Ind. Eng. Chem., Anal. Ed.*, **7**, 218 (1935).

<sup>4</sup> H. Alber, *Mikrochemie*, **14**, 228 (1933).

<sup>5</sup> A demonstration of the use may be found in A. A. Benedetti-Pichler, *Ind. Eng. Chem., Anal. Ed.*, **9**, 149 (1937). A microsectioner for paint films has been described by R. G. Gettens, *Tech. Studies, Field Fine Arts*, **1**, No. 1 (1932).

been developed by Chambers,<sup>6</sup> Fitz,<sup>7</sup> Siedentopf and Peterfi,<sup>8</sup> and others. It is understood that high-power objectives have such short working distances that instruments cannot be inserted between the front lens and the object. Therefore, when working with micro-manipulators under high magnification, the specimens are mounted on the underside of a thin cover glass. The tools are brought into action from below the cover glass.

### EXPERIMENT 36

#### Separation of the Components of a Powder<sup>9</sup>

With a camel's-hair brush, a small amount of a mixture of 1 per cent carbon and 99 per cent alumina is dusted on a clean microscope slide. The slide is placed under the microscope, and the particles of the powder are focused with a low-power objective which has a working distance of at least 20 mm. If observed with reflected light against a colored background, the few black particles may be distinctly seen among the large number of white ones.

Some eight or ten of the black particles are removed from the mixture and collected on another slide within a small area. A sewing needle mounted in a handle, a platinum wire of 0.05- to 0.1-mm diameter, or a glass thread may serve for the transfer of the particles. A suitable glass thread is obtained by drawing out one end of a glass rod to a diameter of 1 mm, and again drawing out the thin part of the rod at a distance of 1 cm from the taper to a thread of 0.05-mm diameter. The thread is broken off so as to leave 1 cm of it attached to the glass rod.

By means of a platinum loop, a small drop of distilled water is placed on the center of a clean slide. To prevent evaporation, the drop is covered with a 1-inch watch glass, and the slide is set aside for later use.

<sup>6</sup> R. Chambers, in McKlung's *Handbook of Microscopical Technique*, New York, P. B. Hoeber, 1929. The instrument is made by E. Leitz, New York. On an application to microchemistry see A. A. Benedetti-Pichler and J. R. Rachele, *Ind. Eng. Chem., Anal. Ed.*, 12, 233 (1940).

<sup>7</sup> G. W. Fitz, *Science*, 79, 233 (1934). The principle is used by Bausch and Lomb.

<sup>8</sup> H. Siedentopf and T. Peterfi, in Abderhalden's *Handbuch der biologischen Arbeitsmethoden*, Abt. V, T. 2, Heft 5. The instrument is manufactured by C. Zeiss.

<sup>9</sup> F. Emich and F. Schneider, *Microchemical Laboratory Manual*, New York, John Wiley & Sons, 1932.

The powder is examined under the microscope, and a black particle is selected which is not closely surrounded by white ones. By moving the slide, this particle is brought into the center of the field of vision. The point of the needle is now moistened with a trace of glycerin by rubbing some glycerin into the skin on the back of the hand and drawing the point of the needle across the treated area. The hand holding the needle is then rested on the stage of the microscope, and the needle is held inclined at 45 degrees to the horizontal so that the point is about halfway between the slide and the front lens of the objective. While looking into the eyepiece, one moves the needle around until a blurred image of its point appears in the field of vision. Now the point of the needle is brought straight down on the particle to be removed. The particle is touched and then lifted out.

Leaving the hand which holds the needle resting on the stage, with the other hand one exchanges the slide with the powder mixture for the other slide with the drop of water. The watch glass is removed, and the edge of the drop is focused. While the drop is observed through the microscope, the point of the needle is inserted. It will be noticed that the particle floats off immediately when the needle touches the water. The drop is again covered with the watch glass, the powder mixture is brought under the microscope, and another black particle is removed, etc. The procedure is repeated until eight to ten black particles are collected in the drop. If some white particles have been carried along, they may be removed, after evaporation of the water, by the same technique as has been described for the transfer of the black particles.

The black particles which finally remain are located within a small area. If so desired, chemical analysis might be begun without further preliminary work.

Some advice may be added. The needle should always be held as nearly vertical as possible. In this manner it is possible to avoid touching other particles in addition to the one selected. If the working distance of the objective is shorter than 15 mm, it will be necessary to bend the end of the needle. Furthermore, it should be pointed out that too large an amount of glycerin on the needle will defeat its purpose. When the particle is touched, the glycerin flows down over the particle and spreads to a drop on the slide. It is then impossible to remove the particle which floats in the glycerin. Of course, the selection of glycerin and water is arbitrary. As a general rule, the adhesive used for the treatment of the thread must be readily soluble in the liquid in which the particles are collected.

**CHEMICAL WORK ON MICROSCOPE SLIDES**

Up to 1900, microanalysis was practically synonymous with chemical microscopy. Nearly all work was carried out on the microscope slide, and H. Behrens performed complete qualitative analyses in this way. Since his manipulative technique often permits saving of time and possesses high sensitivity under favorable conditions, a relatively large amount of space is given to its description. It should be clearly understood, however, that the efficiency of work on the slide varies widely, depending upon the nature of the substances concerned. The separation of lead and bismuth, Expt. 38, may be considered as a gamma procedure. At other times, Expt. 39, several milligrams of material must be taken to compensate for losses in the course of the procedure, and classification as a centigram procedure appears more appropriate.

The fundamental mechanical operation of inorganic analysis is filtration. In its place, Behrens<sup>10</sup> devised a form of decantation suited for work on the microscope slide, which he called *Abschleppen*, "dragging off" or "drawing off." A glass needle is inserted in the drop, and, by inclining the slide and drawing a channel with the thread, the solution is made to flow away from the precipitate. Widening of the end of the channel to a pond by means of the needle results in the formation of a drop of the solution away from the precipitate. If the solution is sufficiently repelled by the surface of the slide, it is even possible to draw the water of the connecting channel into the new drop by proper manipulation of the needle. Washing is performed in the same manner. Whether or not a clear filtrate is obtained depends on the extent to which the precipitate adheres to the slide. Any form of decantation is impossible, of course, if the precipitate does not settle. On the other hand, precipitates clinging tenaciously to the slide can be washed by simply running water from the wash bottle or faucet over the slide. Aside from these extreme cases, most precipitates divide themselves, part going into the filtrate and washings while the rest remains on the slide. Behrens found, however, that many precipitates can be made to behave by evaporating the mixture of solution and precipitate to dryness. The residue is extracted with the wash liquid, and often the extract can then be drawn off without taking along any precipitate.

<sup>10</sup> H. Behrens, *Anleitung zur mikrochemischen Analyse anorganischer Stoffe*, 2nd ed., Hamburg and Leipzig, Leopold Voss, 1899, p. 21.

The decantation procedure of Behrens may be modified in many ways,<sup>11, 12</sup> and it can be essentially simplified whenever either only the solution or only the precipitate is to be collected.

All experimentation performed on material clinging to a plane or slightly curved surface may be considered work on the slide. Size, shape, and material of the slide are of no importance when the classification of the technique is considered. The limits of identification of the procedures depend upon the dimensions of the areas actually occupied by the matter under investigation; the smaller the areas used, the higher is the sensitivity.

### EXPERIMENT 37

#### Conversion of Silver Chloride to Silver Dichromate,<sup>9, 13</sup> Handling Precipitates and Solutions, Fusion, Electrolytic Reduction

Fused silver chloride is reduced with metallic magnesium in acid solution. The metal is washed free of chloride ion, dissolved in nitric acid, and the solution is precipitated with potassium dichromate.

By means of a measuring pipet, 0.05 ml of silver test solution (10 mg Ag per ml) is deposited near the end of a narrow slide.<sup>14</sup> A large drop of 13 M hydrochloric acid is placed on an ordinary microscope slide and, with a platinum loop, small portions of the acid are added to the drop of silver solution until precipitation of the silver ion is complete, i.e., addition of another portion of the acid no longer causes further precipitation. It is understood that the mixture must be stirred well after each addition of precipitant.

**REMOVAL OF SOLUTION AND WASHING OF PRECIPITATE.** Filter paper or spot test paper is cut into squares of approximately 2-cm edge. The major part of the solution is drawn off as described in the introduction to this section and finally removed with filter paper, Fig. 27a. Then, with the edge of a fresh piece of paper, the precipitate is scraped together into a compact little tablet, Fig. 27b. Whenever the edge of the paper becomes moist and soft, another part of the edge is used. For washing, a large drop of water is placed *near* the precipi-

<sup>11</sup> M. H. Hemmes, *Rec. trav. chim.*, **16**, 369 (1898).

<sup>12</sup> E. M. Chamot and C. W. Mason, *Handbook of Chemical Microscopy*, II, 2nd ed., New York, John Wiley & Sons, 1940.

<sup>13</sup> F. Emich, *Mikrochemisches Praktikum*, Munich, J. F. Bergmann, 1924, p. 88.

<sup>14</sup> Narrow slides, approximately 8 mm by 75 mm, are obtained by cutting standard slides parallel to the long edge. Experience has shown that these narrow strips of glass are less inclined to crack when heated. F. Emich, *ibid.*, p. 19.

tate by means of the wash bottle, Fig. 27c. The slide is then tilted so that the drop flows over the precipitate. Without stirring, the preparation is allowed to stand for a minute, whereupon the wash liquid is removed with filter paper as directed above. One repetition of the washing is sufficient. The washed precipitate must form a small tablet near the end of the slide.

**DRYING AND FUSING.** The slide with the precipitate is held a few centimeters above a microflame; a slight, lateral, oscillatory motion ensures the heating of the entire width of the slide and prevents its cracking. When the precipitate appears dry, the slide is lowered until

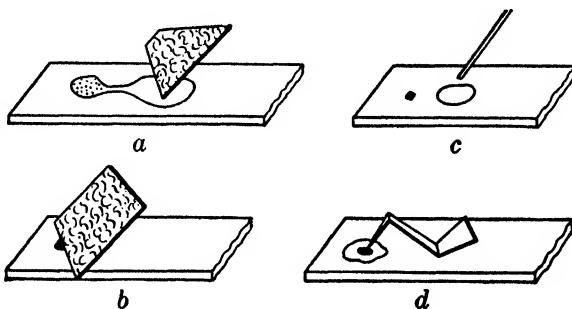


FIG. 27. Working on the slide.

it nearly touches the microflame. Heating is continued until a clear drop of fused silver chloride is obtained (m.p. 450° C). The slide is then placed on a wire gauze or an asbestos board and allowed to cool to room temperature. The fused silver chloride adheres tenaciously to the slide, which simplifies the remaining operations.

**REDUCTION TO METALLIC SILVER.** A large drop of 1 *M* acetic acid is placed on the fused silver chloride. One end of a 3-cm piece of magnesium ribbon is cut to a point; the ribbon is bent into the form of a Z and placed on the slide so that the pointed end touches the fused silver chloride, Fig. 27d. Hydrogen is liberated immediately. When the reduction is complete, the spongy metallic silver floats to the surface of the drop, and the magnesium ribbon is removed.

**REMOVAL OF SOLUTION AND WASHING OF THE SILVER.** Solution and washings are taken up into a capillary pipet. Otherwise the same technique of drawing off is employed as when working with a square of filter paper. The metallic silver adheres to paper, but there is no reason why it should adhere to the pipet rather than to the slide. Of course, the tip of the pipet is too fine to permit passage of the rather large particles of silver. Transfer of the liquid is regulated by prop-

erly inclining the capillary pipet and the slide. Washing twice, with a large drop of water each time, suffices for the removal of chloride ion. Of course, the capillary pipet used for the removal of the solution serves for the removal of the washings, too. Filtrate and washings are rejected.

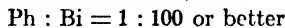
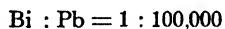
**DISSOLVING THE SILVER AND ADJUSTING THE ACIDITY OF THE SOLUTION.** After washing, the slide is warmed over a Bunsen flame until it is completely dry. Then,  $2\lambda$  of 16 *M* nitric acid is added to the silver by means of a capillary pipet. The mixture is gently heated to hasten the reaction of the metal with the acid. When the silver particles have disappeared, the solution is evaporated just to dryness. The slide is allowed to cool to room temperature. The presence of silver in the residue is now confirmed by either a slide test or a spot test.

**SLIDE TEST.** The residue is dissolved in 5 to 10  $\lambda$  of 2 *M* nitric acid. By means of the capillary pipet used for the addition of the solvent, the solution is transferred to the center of a clean slide and treated with solid potassium dichromate as outlined in Expt. 21.

**SPOT TEST.** The residue is dissolved in 5 to 10  $\lambda$  of distilled water, and the solution is tested as directed in Expt. 12.

### EXPERIMENT 38

#### Separation of Bismuth and Lead<sup>15</sup> Evaporation, Extraction of "Invisible" Residues



Bismuth and lead are quantitatively separated by repeated evaporation of their nitrates with water. Bismuth is hydrolyzed to a water-insoluble basic nitrate. Lead nitrate is extracted from the final residue with water, dilute ammonium acetate, or ammonium nitrate solution.<sup>16</sup>

One milliliter of the bismuth-lead test solution containing 5 mg Bi per ml and 50 mg Pb per ml is diluted with 3 *M* nitric acid to 50 ml and mixed well. Of this solution, 1  $\lambda$  is taken, if slide tests are used for final confirmation. If spot tests are to be employed, it is preferable to start with 3 to 5  $\lambda$  of the solution. The volume is measured with a calibrated pipet, and the solution is transferred to the center of a clean 3-inch watch glass so as to occupy a very small area. A

<sup>15</sup> A. A. Benedetti-Pichler, *Z. anal. Chem.*, 70, 285 (1927).

<sup>16</sup> J. Löwe, *J. prakt. Chem.*, (1) 74, 341 (1858). E. Forche, Dissertation, Leipzig, 1938, established the conditions giving quantitative separation.

square slide of approximately 5-cm edge may be used in place of the watch glass.

**EVAPORATION ON THE STEAM BATH.** A 250-ml Phillips beaker one-third filled with water makes a convenient steam bath for this purpose. When the watch glass is laid on the opening, the steam escapes through the lip. Evaporation of the test drop may be accelerated by blowing a stream of air on the drop as shown in Fig. 28. Cotton in the wide part of the glass tube retains any dust.

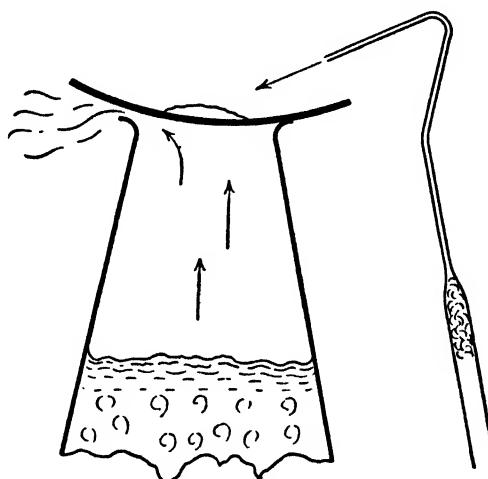


FIG. 28. Evaporation on the steam bath.

When the test drop has evaporated to dryness, 10 to 20  $\lambda$  of water is placed on the residue without removing the watch glass from the steam bath. The residue clings to the glass surface, and it must not be scraped loose with the pipet used for adding the water. It will be easy to cover all the residue with a large drop of water; if necessary, the drop may be spread by means of a glass thread. After complete evaporation of this first drop of water, the treatment with water is repeated four times, which will require not more than a few minutes. The watch glass is then removed from the steam bath, wiped dry at the underside, and allowed to cool to room temperature.

**EXTRACTION OF THE RESIDUE.** Inspection will show a relatively large residue on the watch glass, while the total quantity of lead and bismuth salts is less than 2  $\gamma$ . The residue consists mainly of substances derived from the glass apparatus and the impurities of the reagents. Exclusive use of clear fused quartz in the experiment and in the preparation and storing of reagents would be required to reduce the amount of residue to a minimum.

Whether or not a residue is visible, the area on the slide which was originally occupied by the test drop is treated with 5 to 10  $\lambda$  of distilled water. The solution is spread with a glass thread over the whole area originally covered by the test drop, but care is taken not to loosen any residue. After standing for 3 minutes, the clear extract is taken up into a capillary pipet and set aside with the pipet for future use. Following the above procedure, the residue is further extracted with two 5- to 10- $\lambda$  portions of water.

**FRACTIONAL EVAPORATION OF EXTRACTS AND TEST FOR LEAD.** One gamma of lead, at the most, is contained in the rather large volume of 15 to 30  $\lambda$  of the three extracts. Using always the same spot, only small fractions of the extract are evaporated at a time. Thus, the residue is collected in a small area.

A small fraction of the first extract is placed with the capillary pipet near the end of a microscope slide and evaporated over a micro-flame. While heating, the slide is moved so that the drop describes a circular motion around the tip of the microflame, Fig. 29. In this manner, the slide is heated along a circular zone surrounding the drop, and creeping of the drop is prevented. The slide is removed from the flame when the drop begins to vanish. While the slide is still hot, another fraction of the extract is added to the residue, care being taken that the new drop does not spread beyond the area covered by the residue of the first drop. The evaporation is continued in this way until all the extracts have been used up. Either slide test or spot test may be used for confirming the presence of lead.

**SLIDE TEST.** The final residue is treated with 1  $\lambda$  of 1 mg Cu per ml copper solution (test solution diluted with 9 volumes of water). After evaporation of the copper solution and cooling to room temperature, the residue is treated with nitrite reagent as outlined in Expt. 23.

**SPOT TEST.** The final residue is dissolved in 1  $\lambda$  of water. The solution is taken up in a capillary pipet and tested as described in Expts. 11 and 13.

**SOLUTION OF THE RESIDUE, TEST FOR BISMUTH.** The extracted residue on the watch glass is treated with 10  $\lambda$  6 M nitric acid. Using a glass thread the acid is spread over the area treated in the extractions. By means of a capillary pipet, the acid solution is finally col-

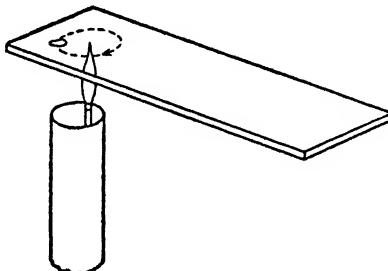


FIG. 29. Evaporation on the slide.

lected as quantitatively as possible and transferred in small fractions to a slide and evaporated. The residue, which must occupy a very small area, is dissolved in approximately 0.5  $\lambda$  or less of 2 *M* nitric acid, added from a capillary pipet.

**SLIDE TEST.** A particle of potassium cobalticyanide of 0.05- $\lambda$  volume (0.5-mm diameter) is added immediately. See Expt. 25.

**SPOT TEST.** The solution is used for the test described in Expt. 14.

### EXPERIMENT 39

#### Separation of Silver, Lead, and Mercurous Mercury,<sup>17</sup> Sublimation, Extraction with Boiling Solutions

Silver, lead, and mercurous mercury are precipitated as chlorides. The mercurous chloride is isolated by sublimation, and lead chloride is extracted from the residue with boiling dilute acid. Acid is required, for hot water cannot dissolve basic chlorides of lead which sometimes form during the sublimation of the mercurous chloride.

**PRECIPITATION OF THE CHLORIDES.** Known solution I contains 10 mg each of silver, lead, and mercurous ions in 1 ml of the solution. Approximately 0.1 ml of this solution is measured off with a pipet and deposited near the end of a narrow slide. About 20  $\lambda$  of 13 *M* hydrochloric acid is placed close to the first drop. The two drops are combined by means of a glass thread, and the mixture is stirred until the precipitate of chlorides has a flocculent appearance. Then the solution is removed with filter paper as described in Expt. 37. The chlorides are washed with two 0.02-ml portions of 2 *M* hydrochloric acid; the washings are removed with filter paper, and the precipitate is collected in as small an area as possible.

**SUBLIMATION OF MERCUROUS CHLORIDE.** The narrow slide with the washed precipitate is held in one hand and a microscope slide of standard dimensions in the other. The microscope slide may rest on the edge of the narrow slide as indicated in Fig. 30a. The precipitate is heated by holding it approximately 2 cm above the point of a microflame. About every 5 seconds the narrow slide is removed from the flame, and the microscope slide is quickly brought up over the residue and held there until the amount of condensate shows no further increase, Fig. 30b. It should be understood that the microscope slide rests on only one edge of the narrow slide; otherwise the two slides are separated by a wedge-shaped air space so that the precipitate is not more than 1 mm below the microscope slide. When con-

<sup>17</sup> N. Schoorl, *Z. anal. Chem.*, **47**, 209 (1908).

densation ceases, the microscope slide is moved back into its former position, Fig. 30a, and the precipitate is heated again. A quickly evaporating condensate of water droplets will be obtained in the first two or three tests. When no more water condenses on the microscope slide, it may be assumed that the residue is dry. The sublimation is now begun by decreasing the distance between the microflame and narrow slide to approximately 5 mm when heating the precipitate for 5 seconds between tests. It is necessary to watch the precipitate carefully and to interrupt heating quickly when white fumes become visible.

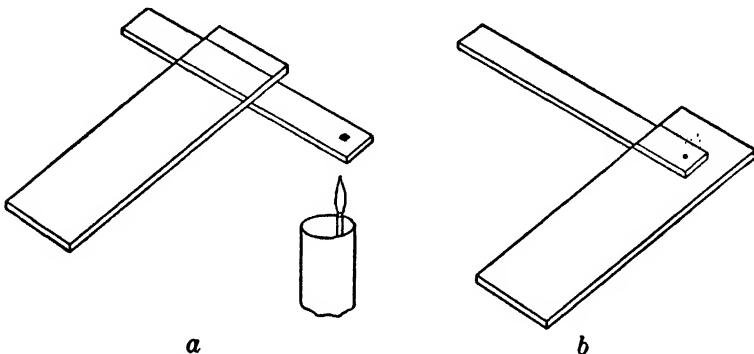


FIG. 30. Sublimation from slide to slide.

ible; the microscope slide is immediately moved up over the precipitate for the collection of the sublimate. The white sublimate of mercurous chloride forms in a circular area above the heated precipitate. When condensation apparently ceases, the microscope slide is drawn back and the precipitate is heated some more. All the sublimate is collected in the same place on the microscope slide and in as small an area as possible. The completeness of the separation is finally proved by heating the residue and then holding over it a fresh portion of the microscope slide. If no sublimate is obtained, it may be assumed that all mercury salt has been removed from the precipitate. A temperature of  $300^{\circ}\text{C}$  suffices for the removal of the mercurous chloride. Excessive heating will cause the residue to fuse and will render the subsequent extraction of the lead chloride difficult.

**IDENTIFICATION OF THE MERCUROUS CHLORIDE.** A platinum loop is used to place a drop of ammonia on part of the sublimate. The moistened portion turns black. The test is easily observed by placing the slide on a piece of white paper.

**EXTRACTION OF THE LEAD CHLORIDE.** The residue on the narrow slide is treated with 0.05 ml of 2 *M* hydrochloric acid. A capillary

pipet with a relatively wide tip is held ready in the right hand for the rapid removal of the extract, and the narrow slide is grasped with the left hand and heated over the microflame. The residue with the dilute acid should be held approximately 3 cm above the flame until tiny steam bubbles start to appear in the drop. The slide is then removed from the flame, and the hot solution is immediately taken up with the capillary pipet. Since the silver chloride adheres to the slide, the procedure presents no difficulties.

**IDENTIFICATION OF LEAD.** The hydrochloric acid extract is transferred in small fractions to a clean slide, and evaporated so as to obtain the residue in as small an area as possible, Expt. 38.

**SLIDE TEST.** The residue is treated with  $5\lambda$  of copper test solution (10 mg Cu per ml), and the mixture is again evaporated to dryness. When the slide has cooled to room temperature, nitrite reagent is applied as directed in Expt. 23.

**SPOT TEST.** The residue is heated close to boiling with approximately  $20\lambda$  of water. The hot solution is quickly taken up into a capillary pipet and transferred to potassium iodide paper as directed in Expts. 11 and 13.

#### **IDENTIFICATION OF SILVER.**

**SLIDE TEST.** The residue of silver chloride on the narrow slide is treated with  $5\lambda$  of 6 *M* ammonia. The mixture is stirred with a glass thread for a few seconds, then quickly taken up with a capillary pipet, and transferred to a microscope slide. After the drop has been covered with a 1-inch watch glass, the directions of Expt. 22 are followed.

**SPOT TEST.** The residue of silver chloride on the narrow slide is dried by heating the slide over a small Bunsen flame so that the silver chloride finally melts. After cooling, an amount of solid potassium cyanide which is approximately twice the volume of the silver chloride is placed on the latter. The slide is now heated over a small Bunsen flame until the potassium cyanide melts and the reduction of the silver chloride is complete. The metallic silver adheres firmly to the slide, and it is washed free from cyanide and chloride by running distilled water over the slightly inclined slide. The slide is finally dried by holding it over a flame. When it has again cooled to room temperature,  $5\lambda$  of 16 *M* nitric acid is added to the silver. Warming over the flame will hasten solution of the metal. The acid is then evaporated, and the residue is dissolved in 5 to  $10\lambda$  of distilled water. The solution is tested as outlined in Expt. 12.

## EXPERIMENT 40

## Test for Ammonium Ion

L.I., 0.1 γ NH<sub>4</sub>

Glass ring.

The test substance is treated with sodium hydroxide. The liberated ammonia is absorbed by a solution of chloroplatinic acid.

**DISTILLATION FROM SLIDE TO SLIDE.** With a capillary pipet, 1 λ of ammonium test solution (10 mg NH<sub>4</sub> per ml) is transferred to the center of one slide, and a like volume of 5 per cent chloroplatinic acid is placed on the center of another slide. By means of a glass ring, both ends of which are carefully polished, a gas chamber<sup>18</sup> is set up

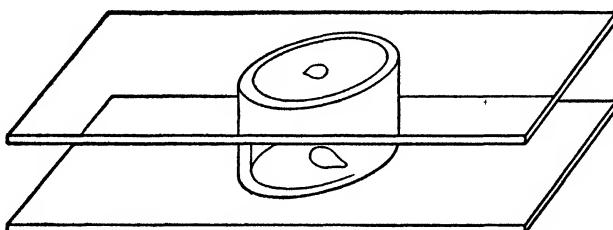


FIG. 31. Gas chamber.

as shown in Fig. 31. Both drops must be inside the chamber, and for most favorable conditions, the drop of chloroplatinic acid must be on the upper slide, since ammonia is lighter than air.

The top slide is lifted, and 1 to 2 λ of 6 M sodium hydroxide is added to the ammonium chloride solution by means of a capillary pipet. The slide with the drop of chloroplatinic acid is immediately replaced. The whole chamber is transferred to the stage of the microscope, and the drop of chloroplatinic acid is focused. Using transmitted light and a magnification of 30 to 80, one may observe the separation of the crystals of (NH<sub>4</sub>)<sub>2</sub>PtCl<sub>6</sub>. As a rule, the yellow tetrahedra and octahedra of the cubic system appear first along the edge of the drop.

<sup>18</sup> F. Emich, *Lehrbuch der Mikrochemie*, Wiesbaden, J. F. Bergmann, 1911, p. 117.

### CHEMICAL WORK IN CAPILLARIES

The technique of working in glass capillaries of 0.3- to 1-mm bore was developed by Emich and his coworkers.<sup>19</sup> In inorganic analysis it is employed to advantage for the performance of certain operations with approximately 1 to 100  $\gamma$  of solid material and a few lambdas of solvent. The relatively large wall surface of capillaries several centimeters long, however, presents disadvantages in the performance of a sequence of separations, and, for this reason, lengthy analytical procedures have never been performed in capillaries of these dimensions. Emich's technique of working in capillaries is, nevertheless, a valuable adjunct to milligram procedures of inorganic qualitative analysis, and it should be acquired before working in either centrifuge cones or microcones.

### EXPERIMENT 41

#### Lead Sulfate, Triple Nitrite, Lead Chromate The Capillary as Adjunct to Work on the Microscope Slide<sup>10, 20</sup>

Approximately 5  $\lambda$  of lead test solution (10 mg Pb per ml) is placed on one slide, and a large drop of 4 *M* sulfuric acid is placed on another slide. Portions of the dilute sulfuric acid are transferred with a platinum loop to the drop of lead solution until the precipitation of lead sulfate is complete. It should be noted that the lead sulfate does not adhere to the slide, and that decantation on the slide would be difficult to perform.

**TRANSFERENCE FROM SLIDE TO CAPILLARY.** Both ends of a capillary of approximately 0.5-mm bore and 10-cm length are cut off squarely. One end of the capillary is held closed with the index finger, and the other end is used to stir up precipitate and solution on the slide. When a rather homogeneous mixture has been obtained, the index finger is lifted off the other opening, and the slurry rises quickly into the capillary.

**SEPARATION OF PRECIPITATE AND SOLUTION.** By means of a pilot flame, a glass bead is fused 3 cm from the dry end of the capillary, Fig. 32a. When the bead cools, contraction of the air inside causes

<sup>19</sup> F. Emich, *Z. anal. Chem.*, **54**, 493 (1915); **56**, 1 (1917); *Monatsh.*, **38**, 219 (1917). A. Fuchs, *Monatsh.*, **43**, 129 (1922). F. Emich and H. Häusler, *Monatsh.*, **53-54**, 335 (1929). F. Emich and F. Schneider, *Microchemical Laboratory Manual*, New York, John Wiley & Sons, 1932, pp. 14, 27, 31, 32, 40, 92, 112, 116, 125, 127, and 129.

<sup>20</sup> F. Emich, *Lehrbuch der Mikrochemie*, Wiesbaden, J. F. Bergmann, 1911, pp. 51, 52.

the slurry to go further into the capillary. All except 1 cm of the empty capillary beyond the glass bead is cut off, Fig. 32b, and the capillary is placed, bead downward, in a centrifuge cone. After swirling in the centrifuge, which will quickly produce a sharp separation of precipitate and solution, Fig. 32c, the capillary is cut at the boundary line so that one piece contains the clear solution and the other the precipitate. The solution, which is not needed in this experiment, is rejected. It is obvious, however, that the described technique is

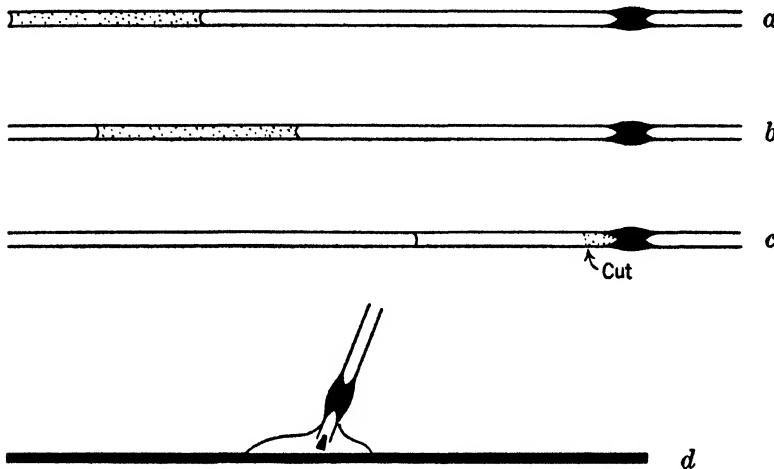


FIG. 32. Separation of solution and precipitate. The bore of the capillary is exaggerated.

equally valuable where the solution is needed rather than the precipitate.

#### TRANSFERRENCE OF PRECIPITATE FROM CAPILLARY TO SLIDE.

(a) PREVIOUS TO SLIDE TEST. Approximately  $5\lambda$  of copper test solution (10 mg Cu per ml) is transferred to a slide. The empty part of the capillary is grasped with fingers or forceps, and the end containing the precipitate is dipped into the copper solution. Without lifting it out of the drop, the capillary is repeatedly tapped against the slide, and the lead sulfate usually leaves the capillary as one lump, Fig. 32d, if the bore of the capillary tapers bluntly at the bead, and if care is taken that no air bubble forms in the capillary. Sometimes it becomes necessary to loosen the precipitate in the capillary by stirring with a fine glass thread.

The mixture of lead sulfate and copper solution is evaporated to dryness, and the residue is treated with nitrite reagent as directed in Expt. 23.

(b) PREVIOUS TO SPOT TEST. Approximately  $5\lambda$  of water is placed on a glass slide, and the precipitate is transferred to this drop as outlined under (a). The mixture of lead sulfate and water on the slide is treated with a crystal of ammonium acetate of approximately  $1\lambda$  volume (1.5-mm diameter). Solution of the lead sulfate may be aided by stirring with a glass thread. The clear solution is treated with  $1\lambda$  of 6 *M* acetic acid and taken up into a capillary pipet. The chromate test is carried out as described for silver in Expt. 12.

### EXPERIMENT 42

#### Recrystallization of Lead Iodide <sup>9</sup>

One large drop of lead test solution (10 mg Pb per ml), one large drop of 0.1 *M* potassium iodide solution, and one large drop of 2 *M*

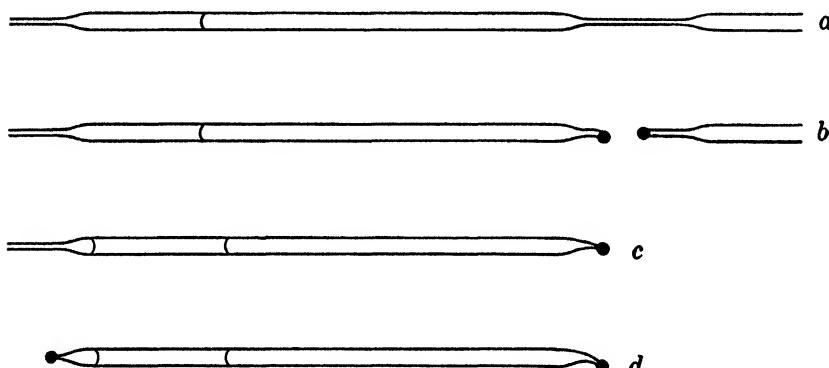


FIG. 33. Sealing capillaries. The bore of the capillary is exaggerated.

acetic acid are placed on a slide so that they do not mix. One end of a capillary of not less than 0.5-mm bore is drawn out to a tip, and portions of the three solutions are taken up into the resulting capillary pipet in the following order: first a 5-mm length of the lead solution, then a 2-mm length of the iodide solution, and finally a 10-mm length of acetic acid.

**SEALING CAPILLARIES.** The capillary is sealed by the method illustrated in Fig. 33. Using a pilot flame or the edge of a Bunsen flame, one draws out the capillary at a distance of 6 cm from the drop, Fig. 33a. The fine capillary obtained is immediately fused off at the point of the taper, Fig. 33b. When the sealed end cools, the solution at the tip is drawn into the tube, Fig. 33c. The now empty tip is broken close to the taper, and the opening is sealed by touching it to the edge of a Bunsen flame, Fig. 33d.

**MIXING.** The end of the capillary which was sealed first is now cut open, and the contents are carefully mixed with a fine glass thread which has a small bead at its end. The capillary is sealed again after being drawn out near the opening. The reduction of the length of the capillary in this operation is negligible if a short piece of thin glass rod is first fused to the opening of the capillary so as to serve as a handle.

**HEATING LIQUIDS IN CAPILLARIES.** The capillary is placed in a 6-inch test tube, and water is added to submerge the capillary completely. The water in the test tube is heated and kept boiling until

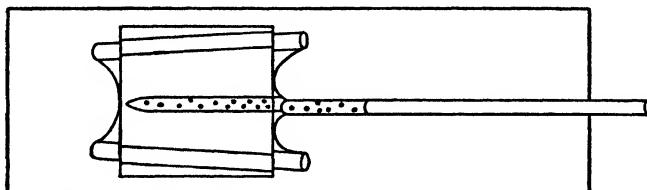


FIG. 34. Cell improvised for examination of the contents of the capillary. The bore of the capillary is exaggerated.

all the lead iodide in the capillary has dissolved. Then test tube and contents are set aside and allowed to cool to room temperature. The slow crystallization of the lead iodide will produce some large hexagonal plates.

**EXAMINATION OF CONTENTS OF CAPILLARY.** The water is poured out of the test tube. Then the capillary is removed, placed on a slide, and examined under a magnifying glass or microscope with both transmitted and reflected light. The objective magnification should not be higher than 8. Optical conditions may be improved by immersing the end of the capillary which contains the precipitate in water. A suitable cell may be improvised with two glass rods of 2-mm outer diameter and a cover slip, Fig. 34.

Finally, the capillary is cut open at both ends. The contents are blown out on a slide and examined again.

### EXPERIMENT 43

#### Isolation of Metallic Mercury, Conversion to Iodide <sup>21</sup>

L.I., 0.2 γ Hg  
Hg : Ag = 1 : 100

Metallic mercury is precipitated by inserting a copper wire in the solution of the salt. The amalgamated copper wire is heated in a capillary, and a condensate

<sup>21</sup> F. Emich, *Z. anal. Chem.*, 54, 500 (1915).

of droplets of mercury is obtained. Exposure to iodine vapors causes conversion of the metal to mercuric iodide.

Approximately 1  $\lambda$  of mercuric test solution (10 mg Hg per ml) is taken up with a platinum loop. If no microscope is available for the inspection of the test, a ten times larger volume should be taken. The solution is transferred into a capillary pipet, made from capillary of approximately 0.5-mm bore, by touching the tip to the loop. The capillary is sealed as outlined in the preceding experiment and illustrated by Fig. 33.

The end of the capillary which was sealed first is cut open, and a 1-mm length of clean, bright copper wire of 0.1-mm diameter is introduced by means of forceps. The capillary is placed, sealed end down, in a cone and centrifuged briefly; this transfers wire and solution to the sealed end. The open end of the capillary is fused shut, and the sealed capillary is thrown into a test tube containing sufficient water for complete immersion. The test tube is heated, and the water is kept boiling for at least one minute. Finally, the hot water is poured out of the test tube; the capillary is removed, placed on a piece of filter paper, and cut so that the wire becomes accessible.

Previous to the isolation of the mercury by distillation, the wire is washed on the filter paper by adding a large drop of water from the wash bottle. When the water has been absorbed by the paper, the wire is picked up with clean forceps and transferred to a piece of dry filter paper. All the moisture is removed by pressing the wire gently between layers of the paper, and then the wire is introduced into a capillary of about 0.5-mm bore and 7-cm length, which is sealed at one end.

This capillary, which is used for the distillation of the mercury, must not contain any moisture. It is, therefore, prepared from a freshly drawn capillary approximately 15 cm long. To prevent flame gases from entering the capillary, the *center* portion of the capillary is heated, drawn out, and fused shut. Two dry capillaries, sealed at one end, are obtained in this way. The amalgamated wire is introduced into the opening of one of them with forceps and brought to the sealed end either by tapping the capillary on the table or by means of the centrifuge, Fig. 35a.

For the distillation, the sealed end of the capillary containing the wire is held into the edge of a non-luminous Bunsen flame so that the rest of the tube is not heated. The heating is continued until a bead of glass forms and completely encloses the copper metal, Fig. 35b. The mercury distils off the wire and condenses in small drops near the

heated portion of the capillary. For the examination of the distillate, the capillary is placed on a slide. Using a magnification of 20 to 50, one first focuses the glass bead, and then examines the whole length of the capillary. In transmitted light, the drops of mercury appear as entirely black, circular disks; it is advisable to cup the hands around the stage of the microscope so as to exclude all light coming from above the stage. With reflected light and a dark background, the silvery color of the metal and the mirror action of the surface of the drops are shown. Both types of illumination are necessary for the

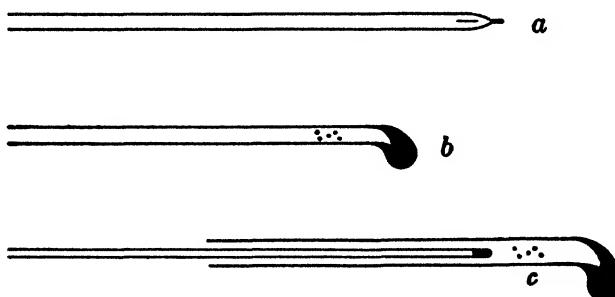


FIG. 35. Distillation of mercury. In *c* the bore of the capillary is very greatly exaggerated.

certain identification of metallic mercury; reflected light is valuable in locating the distillate.

The directions for examination by means of a microscope apply, in a general way, for a magnifying glass. Holding the capillary in front of a lamp produces the effect of observation with transmitted light. For observation with reflected light the capillary is placed on dull black paper or cloth.

In the conversion of the metal to the iodide, a capillary of 0.3- to 0.4-mm outer diameter is used for introducing iodine into the capillary containing the distillate. One end of the fine capillary is pushed against the bottom of the reagent vial containing powdered iodine, causing a small quantity of the reagent to enter the opening of the tube. The fine capillary is then inserted in the wider one containing the distillate so that the iodine is brought close to the drops of mercury, Fig. 35c. The combination is placed on a slide and, by reflected light, the mercury drops are observed with the microscope or magnifying glass at time intervals of several minutes. Depending on the temperature of the room, the conversion to yellow and orange  $HgI_2$  proceeds more or less slowly. The drops become coated with a film of the iodide. Sometimes the coating bursts and liquid mercury is

exuded; the freshly exposed surface of the metal reacts with the iodine vapor, and thus the string and sausage forms of the exudations are produced.

### EXPERIMENT 44

#### Bettendorff's Test <sup>22</sup>

L.I., 0.02 γ As  
As : (Sb, Sn) = 1 : 10,000

The following test is quite selective for arsenic; selenium, tellurium, mercury, and noble metals interfere, since they too are reduced to the elementary state.

Approximately 1 λ of arsenate test solution (10 mg As per ml) is measured off with a platinum loop and then taken up into a capillary pipet of about 0.5-mm bore in the wide part. Large drops of stannous chloride reagent and of 13 M hydrochloric acid are deposited for immediate use on a slide. The point of the capillary pipet containing the arsenate solution is first dipped into the stannous chloride reagent and then into the hydrochloric acid. Approximately 1 λ of the former and 6 λ of the latter are permitted to enter the pipet. The estimation of volume may be based on the length of capillary filled by the drop of arsenate solution; another possibility is the use of the platinum loop for measuring and transferring the reagents. The hydrochloric acid is taken into the pipet last, since it does not contain solid matter and is, therefore, suited for the rinsing of the tip previous to sealing.

Both ends of the capillary pipet are sealed by the standard procedure described in Expt. 42, and the contents are mixed by centrifuging them two or four times from one end of the sealed capillary to the other. The capillary is then placed in a clean, dry test tube, and approximately 2 ml of amyl alcohol (b.p. 130°) is added. The amyl alcohol in the test tube is boiled over a small Bunsen flame for 3 minutes so that the ring of condensate remains approximately 5 cm below the opening of the test tube while the upper part of the capillary is entirely surrounded by the vapor of the boiling alcohol. After heating, the test tube is set aside for 2 minutes. Finally the amyl alcohol is poured back into the reagent bottle, and the capillary is removed from the test tube. The precipitate formed is centrifuged to one end of the capillary, preferably the one which tapers to a finer point. The capillary is placed on a slide and the precipitate is viewed with a magnifying glass or a microscope (magnification 20 to 30) with reflected light and a white background. Immersing the capillary in water,

<sup>22</sup> H. Scheucher, *Monatsh.*, **42**, 411 (1921).

Fig. 34, considerably improves the optical conditions. The elementary arsenic usually shows a characteristic brown color. The capillary with the precipitate is needed in the following experiment.

### EXPERIMENT 45

#### Oxidation of Elementary Arsenic to Arsenic Acid Carius' Treatment in Capillaries<sup>23</sup>

##### Goggles

SEPARATION OF PRECIPITATE AND SOLUTION. The capillary containing the arsenic precipitate is cut open at the empty end. If necessary, the capillary is whirled again in the centrifuge to collect the precipitate as a compact mass in the point of the tube. The supernatant solution is then removed by means of a "contraction pipet,"<sup>24</sup> Fig. 36,

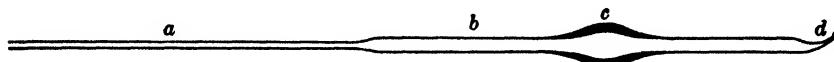


FIG. 36. Contraction pipet. The bore of parts *a* and *b* is exaggerated.

which can be prepared quickly from the pipets with elongated bulbs obtained as a by-product in the drawing of capillaries. The bulb *c* is heated in a Bunsen flame. While the bulb is still hot, the fine capillary *a* of approximately 0.2-mm outer diameter is inserted in the solution above the arsenic precipitate. As the bulb cools, the air inside contracts and draws the liquid into *b*. The tip of the pipet is finally brought close to the precipitate in the capillary. When the solution is completely removed, the pipet is slowly withdrawn from the capillary. The contents of the pipet are rejected by heating the bulb *c*. Any solution which may have remained in the capillary is collected above the precipitate by means of the centrifuge and again removed with the contraction pipet.

WASHING THE PRECIPITATE. A large drop of distilled water is placed on a microscope slide. The capillary is grasped at the sealed end, and the empty part of it is heated by drawing it through a Bunsen flame. The opening of the capillary is quickly inserted in the drop of water. As the glass cools, the water enters the capillary. Brief swirling in the centrifuge transfers the water to the sealed end of the tube, whereupon the contents are thoroughly mixed by means of a glass thread with a small bead at the end. After the precipitate

<sup>23</sup> F. Emich, *Z. anal. Chem.*, **56**, 10 (1917).

<sup>24</sup> A. A. Benedetti-Pichler, *Ind. Eng. Chem., Anal. Ed.*, **2**, 309 (1930).

has been centrifuged into the point of the tube, the wash liquid is removed with the contraction pipet. The washing is repeated once, special care being taken to remove the second wash liquid as completely as possible.

**CARIUS' TREATMENT.** A large drop of concentrated nitric acid is placed on a glass slide. The empty part of the capillary is heated briefly. The opening of the capillary is inserted into the acid, and approximately  $2\lambda$  of the acid is allowed to enter the tube. The acid is transferred to the precipitate by means of the centrifuge, and the opening of the tube is sealed.

The sealed capillary is placed in a clean, dry test tube of Pyrex glass and allowed to slide to the bottom of the test tube. To avoid serious consequences in the event of an explosion, it is advisable to keep the test tube pointed toward a wall as long as the capillary remains sealed. The test tube is held in an almost horizontal position. The part containing the capillary is heated with a nearly luminous Bunsen flame 2 cm in height. The test tube is moved backwards and forwards over the flame so that the entire length of the capillary is heated uniformly. This keeps the nitric acid in continuous contact with the arsenic. The temperature need not exceed  $250^{\circ}\text{C}$ . When the precipitate is no longer visible, the test tube is allowed to cool to room temperature. Using goggles for the protection of the eyes, one allows the capillary to slide into a centrifuge cone. Brief centrifuging suffices to collect the contents of the capillary tube at one end. The tube is removed from the centrifuge cone and cut open in the middle. The contents are taken up into a clean contraction pipet and transferred to the center of a 3-inch watch glass, which is then placed on a steam bath. The drop of acid solution is evaporated to dryness.

#### TEST FOR ARSENIC ACID.

**SLIDE TEST.** The residue on the watch glass is dissolved in  $5\lambda$  of  $2\text{M}$  nitric acid. The solution is transferred to a microscope slide and treated as directed in Expt. 29.

**SPOT TEST.** The residue on the watch glass is dissolved in  $2\lambda$  of  $0.5\text{M}$  nitric acid. The solution is taken up into a capillary pipet and tested as directed in Expt. 17.

## EXPERIMENT 46

Cupric Ammonia Complex, Coloroscopic Capillary<sup>25</sup>

L.I., 1 γ Cu

Approximately 1  $\lambda$  of copper test solution (10 mg Cu per ml) is measured off with a platinum loop and then taken up by a capillary pipet of approximately 0.5-mm bore in the wide part. A drop of 6 *M* ammonia is placed on a slide, the point of the capillary pipet is inserted, and a column of ammonia 3 to 4 cm long is permitted to enter

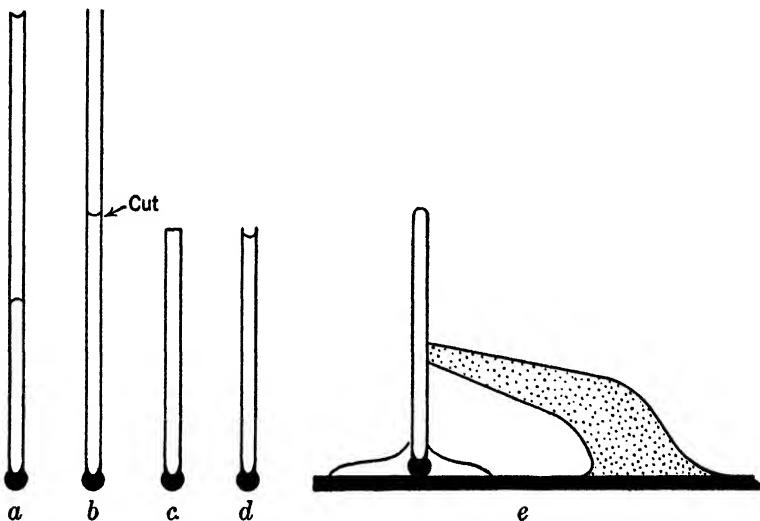


FIG. 37. Coloroscopic capillary. The bore of the capillary is exaggerated.

the pipet. The wide end of the pipet is now sealed as follows. The glass is heated with a microflame 3 cm from the meniscus, drawn out to a finer bore, and immediately sealed shut near the part containing the solutions; the empty part of the pipet is drawn off and rejected. The seal cools to room temperature in a fraction of a minute, and at the same time the solution is drawn into the wide part of the capillary. The tip of the pipet is now empty and is sealed by heating it in the microflame. The contents of the capillary are now mixed by centrifuging the liquid several times from one end of the capillary to the other. For this purpose, the capillary is placed in a cone, briefly cen-

<sup>25</sup> F. Emich and J. Donau, *Monatsh.*, **28**, 825 (1907); **29**, 333 and 959 (1908); F. Emich, *Z. anal. Chem.*, **54**, 496 (1915); H. K. Alber, *Mikrochemie*, **14**, 228 (1933-34); *Ind. Eng. Chem., Anal. Ed.*, **10**, 348 (1938).

trifuged, removed from the cone, re-inserted with the empty end down, centrifuged, etc. At last the contents are centrifuged thoroughly toward the fine point of the capillary so as to collect any precipitate formed in this point. The capillary is placed on a slide and the point is investigated under the microscope or magnifying glass for the presence of a precipitate. The next step is the cutting open of both ends of the capillary. The empty side is opened first; when opening the end containing the solution, care is taken to cut off so

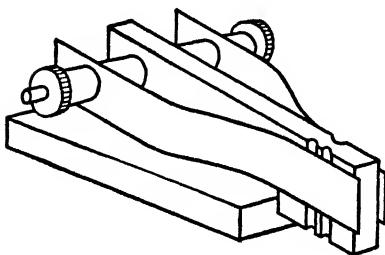
much of the tip that the entire precipitate, if any, is discarded with the tip.

The dry end of the capillary is closed again by rotating it in the edge of a small Bunsen flame until a well-rounded seal has formed. During this operation the capillary is held inclined at 45 degrees with the full end down. Usually a small glass bead is obtained as shown in Fig. 37a. A pointed seal, however, would be objectionable.

FIG. 38. Capillary clamp, approximately natural size. H. K. Alber, *Ind. Eng. Chem., Anal. Ed.*, 10, 348 (1938).

The solution is now transferred to the sealed end by means of the centrifuge, and the capillary is cut evenly just below the meniscus of the solution, Fig. 37b and c.

For observation, the capillary is quickly mounted in vertical position on a square slide of 5-cm edge by means of beeswax, Plasticine, Fig. 37e, or a special capillary clamp, Fig. 38. The end of the capillary must touch the slide and is immersed in a drop of water or cedar-wood oil, Fig. 37e, so as to improve the optical connection between the slide and the capillary. The slide with the capillary is placed on the stage of the microscope, and, with a magnification of 20 to 50, the upper, open end of the capillary is focused. Special care must be taken not to scratch the front lens of the objective. The mirror of the microscope is adjusted until the circular bore of the capillary is brightly illuminated and shows a distinct blue coloration. From time to time it is necessary to add some water by means of a platinum loop to the opening of the coloroscopic capillary in order to maintain either a plane or a convex liquid-air interface, Fig. 37c and e. If, because of evaporation, the meniscus becomes concave, Fig. 37d, observation of the coloration is rendered impossible because of the refractive phenomenon produced; the microscopic image of the bore of



the capillary turns gray or black, depending upon the curvature of the concave meniscus.

The coloration produced by the tetramminocupric ion can be observed with the unaided eye. Of course, the principles of illumination and observation outlined for microscopic observation must be applied.

### THE LIMIT OF IDENTIFICATION OF TESTS BASED ON COLOR

The limit of identification of tests based on the appearance of colorations depends upon the presence of a sufficient quantity of colored matter in the path of the light which is used for the observation of the color. Beer's law may be applied in the form<sup>26</sup>

$$I = \frac{m}{A}$$

in which  $I$  stands for the intensity of the coloration,  $m$  for the absolute mass of colored substance, and  $A$  for the cross-section area exhibiting the coloration. When the test is carried out once on a large scale and once on a small scale, the same coloration is obtained in both instances, if the ratio  $m/A$  is kept constant. This statement applies to the cross-section area  $A$  at the specimen itself, but not to the cross-section area of enlarged images obtained by means of optical apparatus. The composition (color) of the light emerging from the specimen is not altered by properly designed lenses, and the color is not affected by the degree of magnification of the images.<sup>27</sup> Of course, the brightness of the image is inversely proportional to the square of the linear magnification. If, however, the intensity of the light used for the illumination of the specimen is stepped up accordingly, brightness and color of images of different magnification will be identical.

If it is assumed that 1 mg of some dye is sufficient to impart a just perceptible color to a square piece of cloth of 1-dm edge, one may speak of a limit of identification of 1 mg for the use of cloth of this size. When squares of 1-mm edge are employed, the limit of identification will be  $0.1 \lambda$ . By means of projection apparatus an image of the small square, magnified 1000 times, could be obtained, and, if sufficient light is concentrated on the specimen, the image of 1-m edge will possess the same color and brightness as the large piece of cloth of 1-dm edge.

The outlined principles have been deliberately applied by Emich to improve the limit of identification of color tests (Expts. 32 and 46)

<sup>26</sup> A. A. Benedetti-Pichler and J. R. Rachelle, *Ind. Eng. Chem., Anal. Ed.*, **12**, 233 (1940).

<sup>27</sup> A. A. Benedetti-Pichler, *Z. anal. Chem.*, **73**, 205 (1928), footnote.

and bead tests.<sup>28</sup> Clarke and Hermance<sup>29</sup> utilized them likewise to increase the sensitivity of spot tests. The number of more or less spontaneous applications is too large to permit enumeration.

It is evident that a discussion of the color of very small specimens becomes futile when the dimensions are of the same order of magnitude or smaller than the wavelength of light, 0.4 to 0.8  $\mu$ . The approximate validity of the relation between the limit of identification and the ratio  $m/A$  has been established<sup>28</sup> for drops of 5- $\mu$  diameter;  $A = 0.00002 \text{ mm}^2$ .

### EXPERIMENT 47

#### Limit of Identification of the Ammonia Test for Copper

Graduate cylinder, 50-ml.

6 test tubes, 6-inch.

1 ml copper test solution diluted to 10 ml.

60 ml 3 M ammonia.

Ten milliliters of the diluted copper test solution (1 mg Cu per ml) is treated with an equal volume of 3 M ammonia. After mixing, part of the solution is used to fill a test tube to a height of 40 mm. Ten milliliters of the deep blue solution is diluted to twice this volume with ammonia, etc. The procedure is continued until the dilution is reached which no longer shows a blue coloration in a layer 40 mm thick. The observation is made by holding the test tube over brightly illuminated white paper while looking down along the axis of the tube. The limiting concentration and the limit of identification of the test tube technique are recorded.

Two coloroscopic capillaries of approximately 0.5-mm bore are prepared. Taking the solutions from the test tubes, one fills the first capillary with the solution representing the limiting concentration, and the second capillary with the next dilution which showed absence of the blue coloration in the test tube. Both coloroscopic capillaries are finally cut to a length of 40 mm, whereupon the contents are tested for presence of a coloration. If necessary, the test is repeated with other dilutions. The limiting concentration is recorded and compared with the one obtained in test tubes. The inner diameter of the test tube is measured with a millimeter rule, and the limit of identification obtainable with a coloroscopic capillary of 0.1-mm bore is calculated.

<sup>28</sup> J. Mika, *Kolloidchem. Beihefte*, 23, 309 (1927).

<sup>29</sup> B. L. Clarke and H. W. Hermance, *Ind. Eng. Chem., Anal. Ed.*, 9, 292 (1937).

CENTIGRAM PROCEDURES <sup>30</sup>

## WORK IN CENTRIFUGE CONES

Approximately 10 mg of solid sample (5 mg of alloys) is taken, when lengthy separations are to be carried out. Most of the work is performed in 3-ml centrifuge cones shown in Fig. 4A. For the adding of reagents may be used measuring pipets of 2-ml capacity divided in 0.1 ml and measuring pipets of 0.2-ml capacity divided in 0.01 ml, which are easily cleaned by the suction device shown in Fig. 14B. The work in centrifuge cones is extremely simple. Precipitates are collected in the points of the cones by means of the centrifuge, and with a powerful electric centrifuge the precipitates become so tightly packed into the point that the supernatant solutions may be poured off. Nevertheless, it is better to use the suction-operated siphons shown in Figs. 15A and C, for they permit a sharper separation of solution from precipitate. Only very slight suction should be used, and the tube shown in Fig. 15B will prove very practical for instantaneous breaking of the suction. Especially when the centrifuge is not very powerful, the cones should be held in a perpendicular position, Fig. 15A, while the centrifuge is withdrawn.

If so desired, any or all of Expts. 48 to 55 may be performed on the centigram scale by simply following the directions and using ten times larger masses and volumes. The feasibility of this procedure has been proved by the work of Richard N. Boos.<sup>31</sup> Fusions may be carried out in small crucibles or spoons having a capacity of a few tenths of a milliliter.

DISTILLATION FROM CONE TO CONE <sup>31</sup>

A special device, Fig. 39, which permits quantitative collection of distillate and residue, has been developed for the distillation of arsenic trichloride. The solution to be distilled is placed into the small cone *a* of approximately 2-ml capacity. By means of its handle, this cone is then attached to a glass hook and lowered into the large cone *b* of

<sup>30</sup> One may compare C. J. van Nieuwenburg and Ir. G. Dulfer, *A Short Manual of Systematical Qualitative Analysis*, Amsterdam, D. B. Centen's Uitg. Maatschappij, 1933; C. J. Engelder, T. H. Dunkelberger, and W. J. Schiller, *Semi-Micro Qualitative Analysis*, New York, John Wiley & Sons, 1936; P. Arthur and O. M. Smith, *Semi-Micro Qualitative Analysis*, New York, McGraw-Hill, 1938; and numerous other books on semi-micro analysis.

<sup>31</sup> R. N. Boos, Master's Thesis, New York University, 1940.

approximately 12-ml capacity so that the rim of the small cone comes to rest on the indentations projecting into the interior of the large cone. The small cone has a graduation mark at 0.15 ml. Both cones either must be dry or must be rinsed with concentrated hydrochloric acid before use in the separation of arsenic.

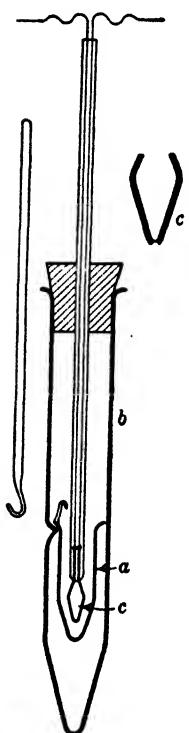


FIG. 39. Distillation from centrifuge cone to centrifuge cone. Approximately half natural size.

The large cone is immersed in ice water up to the cork, and the distillation is started by supplying just enough current to produce slow steady boiling of the solution in the small cone. The point of the V-shaped wire must always remain immersed. To finish the distillation, the current is shut off, and the apparatus is left in the ice water for 2 minutes. Then the heater is removed, and the large cone is centrifuged with the small cone still in place so as to collect all the distillate which adheres to the inside walls of the large cone and to the outside wall of the small cone. The small cone, which contains the residue in its point, is then lifted out of the large cone by means of a glass hook. Residue and distillate may be transferred to 3-ml centrifuge cones by means of the siphon.

## MILLIGRAM PROCEDURES

### WORK IN MICROCONES<sup>10, 88</sup>

Approximately 1 mg of solid substance is taken as sample when lengthy separations have to be carried out as in the general scheme

<sup>82</sup> F. E. Blacet and D. H. Volman, *Ind. Eng. Chem., Anal. Ed.*, **9**, 44 (1937).

<sup>88</sup> F. Emich, *Lehrbuch der Mikrochemie*, Wiesbaden, J. F. Bergmann, 1911.

for the detection of cations. Most of the work is performed in microcones of 0.7- to 1-ml capacity, Fig. 4B, which have proved highly efficient as long as the total volume of solution remains above 10  $\lambda$  and the mass of precipitates does not drop below 5  $\gamma$ . Aside from calibrated capillary pipets, measuring pipets of 0.2-ml capacity divided in 0.01 ml are used for adding reagents.

### SEDIMETRIC ESTIMATION OF QUANTITY<sup>34</sup>

Purely qualitative findings have hardly any value. Quite reliable<sup>35</sup> estimations may be obtained by a comparison of the volumes of precipitates which have been collected in the point of cones by means of the centrifuge. It need hardly be mentioned that the "volume" of a precipitate depends largely upon the size of the particles, the strength of centrifugal force, and the time of centrifuging. Exact reproduction of the volume of precipitates is very difficult,<sup>36</sup> but approximate estimation of the volume of a small quantity of crystalline precipitate is quite simple. Gelatinous precipitates, however, always cause considerable uncertainty.

The straight-tip cones, Fig. 4C, offer obvious advantages in the estimation of volume.<sup>34</sup> The lower portion is made of thick-walled capillary tubing of uniform bore. A bore of 2 mm is suited for the estimation of the volume of large precipitates. For small volumes a bore of 1 mm is recommended. To assure ease of cleaning, the capillary part of these cones should not be longer than 20 to 25 mm.

## EXPERIMENT 48

### Estimation of the Quantity of Silver

Four microcones of the type shown in Fig. 4B, the points of which are as nearly identical as possible in shape and dimensions, are selected. By means of a calibrated capillary pipet, measured volumes of silver test solution (10 mg Ag per ml) are transferred to three of these cones. The first microcone receives 1  $\lambda$  of the solution, the second 5  $\lambda$ , and the third 10  $\lambda$ . The tip of the pipet is touched to the

<sup>34</sup> A. A. Benedetti-Pichler and W. F. Spikes, *Introduction to the Microtechnique of Inorganic Qualitative Analysis*, Douglaston, N. Y., Microchemical Service, 1935.

<sup>35</sup> H. K. Alber and C. J. Rodden, *Ind. Eng. Chem., Anal. Ed.*, **10**, 47 (1938).

<sup>36</sup> I. M. Kolthoff and E. B. Sandell, *Textbook of Quantitative Inorganic Analysis*, New York, Macmillan, 1936, p. 640. H. J. Hamburger, *Biochem. Z.*, **1**, 259 (1906); **71**, 415 (1915); **74**, 414 (1916); *Z. anal. Chem.*, **56**, 95 (1917).

inside wall of the microcones just below the rim, and the pipet is emptied by blowing with the mouth. The solutions are collected in the points of the cones by centrifuging briefly. After the pipet has been cleaned and dried,  $10\lambda$  of an unknown silver solution<sup>87</sup> is transferred to the fourth microcone in a like manner.

The contents of each microcone are treated as follows. By means of a 0.2-ml measuring pipet 0.02 ml of 0.1 *M* hydrochloric acid is added. After brief centrifuging, the contents are heated on the steam bath, Fig. 7, and agitated by means of a glass thread, Fig. 4E, until the supernatant solution has become practically clear. Care must be taken not to bring any precipitate up on the walls above the solution. The stirrer is finally removed, and the precipitate is collected in the point of the tube by means of the centrifuge. Equal force and duration of whirling are best assured by centrifuging all four precipitates simultaneously, if the centrifuge permits this. Sometimes some of the precipitate remains on the surface of the solution or on the walls of the tube. One may try to stir these particles of the precipitate into the solution by means of the glass thread without disturbing the precipitate in the point. Whirling will then throw the particles into the point. Rinsing the walls down with 1 or 2 small drops of ethyl alcohol may assist in the removal of precipitate from the surface of the solution.

The amount of silver in the unknown solution is estimated by holding the microcones side by side and comparing the volumes of silver chloride. The concentration of the silver ion in the unknown solution is expressed as gram silver ion per liter solution. The experiment may be repeated with the use of straight-tip cones of 1-mm bore of the measuring capillary.

All the cones are cleaned immediately.

## EXPERIMENT 49

### Separations in the Microcone

Mercury and lead are precipitated as sulfides after careful adjustment of the acidity of the solution. The sulfides are treated with dilute nitric acid. The remaining mercuric sulfide is dissolved in nitric-hydrochloric acid mixture and reprecipitated for estimation of the quantity. The lead is isolated as the sulfate and converted to the chromate for the purpose of estimation of the quantity.

**MEASURING THE SOLUTION WITH THE CENTRIFUGAL PIPET.** Exactly  $10\lambda$  of known solution II is taken up into a centrifugal pipet. The outside of the tip is wiped dry with a piece of filter paper, and the

<sup>87</sup> See Appendix.

pipet is inserted in a microcone as shown in Fig. 21 and briefly whirled in the centrifuge. The contents of the pipet are thus quantitatively transferred into the point of the cone.

By means of a 0.2-ml measuring pipet, 0.04 ml of distilled water is added. The microcone is placed on the steam bath, and 10  $\lambda$  of 3 M ammonium chloride is added to the hot solution with the measuring pipet. By means of a glass thread, the contents of the microcone are mixed without removing the cone from the steam bath. The thread is finally withdrawn, and the microcone is briefly centrifuged so as to collect all the solution in the tip. The microcone is best returned to the steam bath to keep its contents hot for the following treatment with hydrogen sulfide.

**PASSING GAS INTO SOLUTIONS.** One end of a piece of soft-glass tubing of 6-mm bore is drawn out to a capillary of approximately 1-mm bore and 10- to 20-cm length. A plug of cotton is inserted into the wide part of the tube to serve as filter for the gas. The end of the wide capillary is then drawn out to a fine capillary of approximately 0.1-mm bore, which is broken off so as to give it a length of 10 cm. By means of a cork over the wide part and a buret clamp, the tube is fixed in a perpendicular position, Fig. 40, and connected to a hydrogen sulfide line which supplies the gas under a pressure corresponding to a column of water of at least 30 cm. The flow of gas is started, and when the odor of hydrogen sulfide is perceptible at the exit of the fine capillary, the microcone is taken from the steam bath, and the fine capillary is inserted into the hot solution, Fig. 40. A stream of very small gas bubbles is obtained, and there is no danger that the solution could be thrown out of the cone.

Hydrogen sulfide is passed into the solution until the solution has cooled to room temperature. Without the stream of hydrogen sulfide being turned off, the microcone is removed from the capillary and cooled by running tap water over the outside. Then 0.05 ml of distilled water is

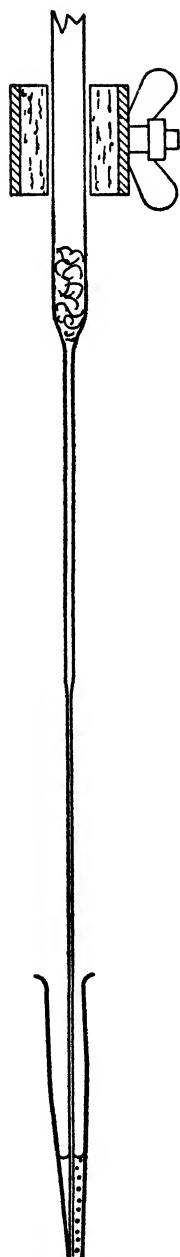


FIG. 40. Passing gas into solutions. Bore of fine capillary is exaggerated.

added from the measuring pipet, and after centrifuging briefly, the cold mixture is saturated with hydrogen sulfide. If the microcone is raised sufficiently so that the opening of the delivery capillary touches the bottom of the microcone, the precipitate is mixed with the solution by the stirring action of the stream of bubbles.

The microcone with the precipitate is set aside, and the end of the fine delivery capillary is carefully wiped dry with filter paper before the supply of gas is shut off.

**APPROXIMATE ESTIMATION OF THE MASS OF METALS.** The estimation of the amount of metals in the sulfide precipitate may be based on the experience that like masses of metals of the hydrogen sulfide group give sulfide precipitates of nearly identical volume.<sup>34</sup>

Exactly  $5 \lambda$  of mercuric test solution (10 mg Hg per ml) is measured off with a calibrated capillary pipet and transferred to a microcone, the tip of which closely resembles the tip of the microcone containing the sulfide precipitate. Then 0.04 ml of water and 0.01 ml of 3 M ammonium chloride are added, and the treatment with hydrogen sulfide is carried out as described. Finally, before the supply of gas is shut off, the end of the delivery capillary which came in contact with the solution is broken off and rejected.

The two microcones with the precipitates are placed in the centrifuge and whirled for 30 seconds to 1 minute. The volumes of the precipitates are compared, and the absolute mass of metal is estimated and recorded. The precipitate of mercuric sulfide used for comparison contains 50  $\gamma$  of metal (mercury); it is reserved for further use.

**SEPARATION OF A LARGE VOLUME OF SOLUTION FROM A PRECIPITATE.** A capillary siphon, Fig. 41, is obtained from a capillary pipet of 0.5- to 1-mm bore and 15-cm length by bending it to an angle of approximately 60 degrees. The middle of the capillary pipet is cautiously heated in the edge of the non-luminous Bunsen flame and 1 cm above the barrel of the burner. Holding one end of the pipet, one may allow the other to drop slowly by gravity until the desired bend is obtained. The fine capillary at the tip is broken off close to the taper so as to get a reasonably rapid flow of liquid.

The use of the capillary siphon is illustrated by Fig. 41. The leg with the wide opening is placed in the microcone which is to receive the centrifugate. The leg with the tip is then laid in the cone containing the precipitate so that the tip and the wide part of the capillary rest on *opposite* sides of the microcone. The capillary siphon fills by itself, if the tubes are properly inclined. The tip of the siphon is pushed close to the precipitate so that practically all the centrifugate is removed in one operation. The cone with the precipitate is finally

withdrawn. The siphon remains in the microcone with the centrifugate, and the cone is placed in a wooden block in slightly inclined position so that the siphon remains filled.

The microcone containing the precipitate is whirled in the centrifuge, and any centrifugate collecting above the precipitate is removed with the siphon. Siphon and centrifugate are set aside as before and held ready for the collection of the washings.

**WASHING PRECIPITATES.** The sulfide precipitate is washed with 3 portions of  $10 \lambda$  of 0.12 *M* nitric acid. Each time the liquid is trans-

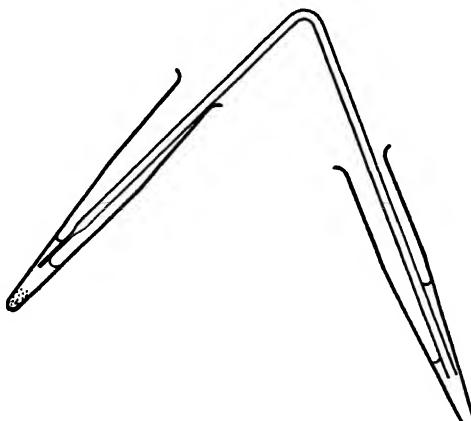


FIG. 41. Use of capillary siphon.

ferred to the opening of the microcone by means of a measuring pipet, and without stirring up the precipitate, the wash liquid is spread over the walls of the cone by means of the glass thread. Then the wash liquid is collected in the point of the cone by brief whirling in the centrifuge, and the precipitate is now stirred up with the glass thread so that none of it is spread over the walls of the cone. After centrifuging, the wash liquid is withdrawn with the capillary siphon.

When the last washing has been collected, the tip of the capillary siphon is broken off, and the contents of the siphon are transferred to the microcone by blowing with the mouth. After addition of 0.01 ml of water, the combined centrifugate and washings are heated on the steam bath, and the hot solution is again treated with hydrogen sulfide to make certain that the precipitation of the sulfides was complete. If no precipitate is obtained, centrifugate and washings are rejected.

**EXTRACTION OF PRECIPITATE.** By means of the measuring pipet 0.02 ml of 3 *M* nitric acid is added to the washed sulfides. The acid

is collected in the point of the microcone by whirling briefly in the centrifuge. The mixture is stirred with a glass thread until the precipitate is evenly distributed in the acid. Then the microcone is placed on the steam bath and heated for 5 minutes while stirring continuously. The formation of plastic sulfur which tends to include sulfides is easily avoided when the above directions are followed. It is obvious that chloride ion must be absent, since it would cause solution of mercuric sulfide.

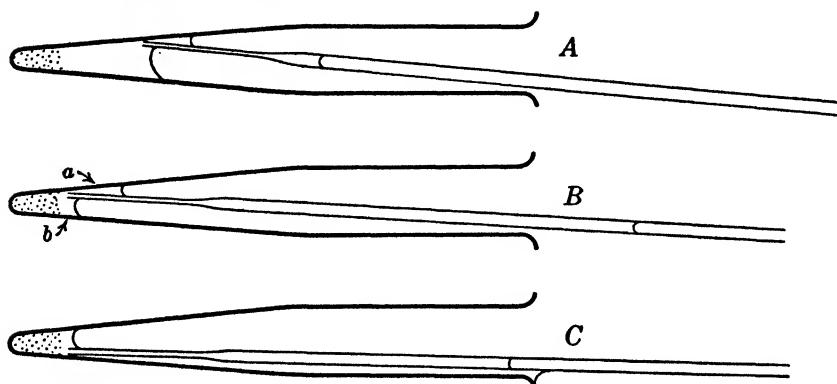


FIG. 42. Separation of solution and precipitate by means of a capillary pipet. *A* and *B* show the correct procedure. The bore of the capillary is exaggerated.

**SEPARATION OF A SMALL VOLUME OF SOLUTION FROM A PRECIPITATE.** The microcone is centrifuged to collect the residue in the point. If some of the residue clings to the walls, it is stirred into the solution and combined with the main portion in the point by means of the centrifuge.

Use of a siphon for the transfer of the solution would be inadvisable, for the small amount of solution would not even fill the siphon. Use of a capillary pipet is preferable. A capillary of 0.5- to 1-mm bore and 20-cm length is selected, and one end is drawn out to a tip of 15- to 20-mm length and not less than 0.2-mm bore. The microcone is held almost horizontal, and the capillary pipet is laid in the cone <sup>34</sup> as shown in Fig. 42A. The rate at which the liquid is drawn into the pipet may be regulated by inclining the cone. The cone is held in the left hand, and the pipet is slowly pushed into the cone with the forefinger of the right hand so that the opening of the tip continuously remains just below the meniscus of the solution in the cone. The tip of the pipet is finally brought to a point 1 mm from the surface of the precipitate, Fig. 42B. When the cone is properly inclined, the liquid is quantitatively taken up by the pipet. Meniscus

*b* disappears first, and very soon afterwards meniscus *a*. Now the capillary pipet is withdrawn from the microcone, and its contents are transferred to a clean, dry microcone by blowing with the mouth. The pipet is saved for transferring the washings. If there is any doubt as to the complete separation of solution and precipitate, the cone containing the precipitate is centrifuged again, and any solution showing above the precipitate is transferred to the main portion of centrifugate as described.

If the capillary pipet is not perfectly straight, or if a straight pipet is laid in the microcone in such a way, Fig. 42C, that a narrow space is formed between pipet and cone, liquid is drawn into this space by capillary attraction and is not taken up by the capillary pipet. When the pipet is withdrawn, the liquid spreads over the walls of the microcone.

**WASHING PRECIPITATES WITH HOT SOLUTIONS.** Since small volumes of hot wash liquid would acquire room temperature before they reach the precipitate, the precipitate must be heated with each portion of the wash liquid.

The residue of the extraction with nitric acid is washed with 3 portions of  $5 \lambda$  of 0.12 *M* nitric acid. Each portion of wash liquid is added by means of a calibrated capillary pipet. The wash liquid is first used for rinsing the walls of the cone with the aid of the glass thread. After centrifuging, the residue is mixed with the wash liquid while heating the cone on the steam bath. The cone is quickly centrifuged, and the washings are withdrawn while the microcone is still warm. The washings are combined with the centrifugate, which is set aside for later use.

**NITRIC-HYDROCHLORIC ACID MIXTURE, SOLUTION OF MERCURIC SULFIDE.** Large drops of concentrated nitric acid and concentrated hydrochloric acid are placed on a glass slide. Approximately  $10 \lambda$  of each acid is taken up into a calibrated capillary pipet, and the contents of the pipet are then blown out on a clean part of the slide so as to mix them. Five lambdas of the mixture is transferred to the residue from the extraction with nitric acid.

The microcone is first centrifuged. Then, with the aid of the glass thread, the residue is mixed with the acids, and the microcone is placed in water at  $40^\circ \text{C}$  for 2 minutes, Fig. 8. The residue will no longer contain darkly colored particles, and the microcone is transferred to the steam bath and heated for 3 minutes while air is blown from a capillary into the cone, Fig. 9.

The contents of the microcone are treated with 0.05 ml 0.3 *M* hydrochloric acid and 0.01 ml 3 *M* ammonium chloride. The mixture

is agitated, warmed on the steam bath, and centrifuged. The clear solution is then separated from the sulfur and transferred by means of a capillary pipet to a straight-tip cone with a measuring capillary of 1-mm bore. The residue is rejected without washing.

The solution in the straight-tip cone is treated with hydrogen sulfide as described at the outset. When the precipitation of the mercuric sulfide is complete, the cone is set aside, and in the meantime the standard precipitate obtained with 50  $\gamma$  mercury in a microcone is transferred to a straight-tip cone to serve as standard for comparison.

**TRANSFER OF PRECIPITATES.** The sulfide precipitate obtained with 50  $\gamma$  mercuric ion is transferred from the microcone to a straight-tip cone having a measuring capillary of 1-mm bore. By means of a glass thread the precipitate is stirred up so as to obtain a slurry in the *tip* of the cone while the main portion of the supernatant solution remains clear. Then a capillary of 0.5- to 1-mm bore and 20-cm length is cut off evenly at both ends. While one end is closed with the finger, the other end is inserted in the microcone so that it touches the bottom of the tip. Cone and capillary are inclined at an angle of 45 degrees to the horizontal, and the finger is taken off the upper opening of the capillary. The liquid rushes into the capillary, the slurry entering first. The top end of the capillary is now closed again with the finger, and the contents are transferred to the straight-tip cone. If the capillary is held in a vertical position for some time before being emptied, the precipitate may collect at the lower end of the capillary, and only part of the solution in the capillary is needed in the final operation of the transfer. Of course, the whole process is to be repeated until all the precipitate is collected in the straight-tip cone.

The precipitates in both straight-tip cones are collected by means of the centrifuge. The mass of mercuric ion in the 10- $\lambda$  portion of known solution II is estimated and recorded. Both cones are stoppered, and the precipitates are reserved for use as standards in the following experiment.

**EVAPORATION IN THE MICROCONE.** The centrifugate containing the lead is now evaporated after addition of sulfuric acid. The solution might be transferred to a watch glass for this purpose. Evaporation in the microcone has the advantage, however, that losses connected with transfers are eliminated. In addition, the evaporation must proceed until fumes of sulfur trioxide appear, and the creeping of the hot sulfuric acid causes less difficulty in the cone than on the watch glass.

The nitric acid extract is treated with 10  $\lambda$  of 6 *M* sulfuric acid. After brief centrifuging, the cone is placed on the steam bath and

heated while a stream of air is blown into the microcone. When the volume of the liquid in the microcone has been reduced to approximately  $3\lambda$  and appears to remain unchanged on further heating, the cone is transferred to a heating block.

**HEATING OF MICROCONES.** The block, Fig. 9, should have a temperature between  $100^\circ$  and  $110^\circ\text{C}$  when the cone is inserted. A gentle current of air is blown into the microcone, and the temperature is rapidly raised (to  $160^\circ\text{C}$  within 5 minutes) until the dense white fumes of sulfur trioxide are observed. Then the microcone is removed from the heating block, and allowed to cool to room temperature.

**PRECIPITATION OF LEAD SULFATE.** The microcone is thoroughly cooled by running tap water over its outside. Then 0.02 ml of water is added at once. With the aid of a glass thread the liquid is used to rinse the walls of the microcone briefly. Then the cone is centrifuged, and immediately afterwards the contents are mixed in the point of the cone by means of the glass thread. The precipitation is set aside for exactly 6 minutes. Then the cone is centrifuged, and the centrifugate is removed with a capillary pipet and rejected.

The lead sulfate is dissolved in 3 *M* ammonium acetate. The reagent is added in portions of  $5\lambda$  until the lead sulfate is dissolved. The mixture is warmed and agitated with the glass thread after each addition of solvent.

**TRANSFER FROM CONE TO CONE.** The solution in the microcone is first collected in the point by centrifuging. The solution is then taken up into a capillary pipet as has been described for the separation of a centrifugate from a precipitate. The contents of the pipet are transferred to a straight-tip cone having a measuring capillary of 2-mm bore. The transfer is made quantitative by rinsing the microcone. Frequently one is able to use for this purpose the reagent solutions which have to be added in the next step of the procedure. In the present instance, the solution is to be diluted, and thus 2 portions of 0.02 ml of distilled water are used for rinsing the microcone. After centrifuging, the rinsings are transferred to the straight-tip cone by means of the capillary pipet used in the transfer of the ammonium acetate solution of the lead sulfate.

**PRECIPITATION OF LEAD CHROMATE, ESTIMATION OF QUANTITY.** The solution in the straight-tip cone is treated with  $5\lambda$  of concentrated acetic acid, mixed with a glass thread, and briefly centrifuged. The cone is placed on the steam bath, and 0.02 *M* potassium dichromate solution is added in portions of  $5\lambda$  volume, with stirring after each addition, until the precipitation is complete and the solution has a distinct orange-red color. The mixture is left on the steam bath for

5 more minutes and stirred from time to time. The cone is then removed from the steam bath and set aside to cool.

By means of a calibrated capillary pipet  $5 \lambda$  of lead test solution (10 mg Pb per ml) is transferred to a second straight-tip cone of like dimensions, treated with  $10 \lambda$  of 3 *M* ammonium acetate,  $5 \lambda$  concentrated acetic acid, and  $40 \lambda$  of water. The precipitation with dichromate is performed as directed above. Both straight-tip cones are finally centrifuged, and the volumes of the precipitates are compared. The amount of lead ion found in  $10 \lambda$  of known solution II is recorded. The standard precipitate may be reserved for the following experiment.

Ten lambdas of known solution II contains  $20 \gamma$  of mercuric ion and  $50 \gamma$  of lead ion, i.e.,  $70 \gamma$  of metals of the hydrogen sulfide group.

## EXPERIMENT 50

### Separation of the Metals of the Copper Group

The copper group will be understood to comprise mercury, silver, lead, bismuth, copper, and cadmium. The scheme of separation is a modification of Swift's<sup>38</sup> semi-quantitative procedure. As an alternative may be suggested a scheme which makes use of Löwe's method for the separation of bismuth and lead, Expt. 38. It is particularly suited for the detection of traces of bismuth.

**PRECIPITATION WITH HYDROGEN SULFIDE.** Precisely  $10 \lambda$  of known solution III is transferred to a microcone by means of a centrifugal pipet. The solution is treated with 0.04 ml of water and 0.01 ml of 3 *M* ammonium chloride. The white precipitate of silver chloride and bismuth oxychloride is left suspended in the solution. The mixture is heated on the steam bath and then saturated with hydrogen sulfide while it slowly cools to room temperature. The contents of the cone are then thoroughly cooled with tap water, and, after addition of 0.05 ml of water, the mixture is again saturated with hydrogen sulfide. Precipitate P11 and solution S11 are separated by means of a capillary siphon, and the precipitate is washed with three 0.01-ml portions of 0.12 *M* nitric acid. The washings are combined with the centrifugate.

**TEST FOR COMPLETE PRECIPITATION.** Centrifugate S11 is treated with 0.01 ml of water, heated on the steam bath, and saturated with hydrogen sulfide. If no precipitate is obtained, the solution is rejected.

<sup>38</sup> E. H. Swift, *A System of Chemical Analysis*, New York, Prentice-Hall, 1939. The adaptation to the milligram scale has been tested by Anne G. Loscalzo, Master's Thesis, New York University, 1941.

If centrifugate S11 is to be analyzed for the presence of ions of the following analytical groups, the solution is immediately evaporated to dryness. This is done by either heating the microcone on the steam bath and blowing air into the cone, or by transferring the solution by fractional evaporation to a watch glass as described in Expt. 38. The residue is then reserved for later use.

If a precipitate is obtained in centrifugate S11 with hydrogen sulfide, the solution is immediately cooled with tap water and then saturated with hydrogen sulfide. The microcone containing the mixture is sealed with a pressure cap, Fig. 10, and then immersed for 15 minutes in boiling water. If a large precipitate forms, saturation with hydrogen sulfide and heating are repeated once more. The tube is cooled and opened. The microcone is centrifuged, and solution and precipitate are separated by means of a capillary siphon. Precipitate P12 is washed with two 5-λ portions of 0.12 *M* nitric acid. The washings are combined with centrifugate S12, which is either rejected or immediately evaporated to dryness as outlined above for centrifugate S11. Precipitate P12 is transferred as a slurry to the microcone containing P11. Approximately three 10-λ portions of 0.12 *M* nitric acid are used in the transfer. When all the liquid and all the precipitate (P11 and P12) have been collected in one cone, the mixture is first thoroughly agitated with a glass thread, and then, after centrifuging, the acid used in the transfer is removed and rejected.

**EXTRACTION OF THE COPPER GROUP WITH NITRIC ACID.** Precipitate P11 or the combined precipitates P11 and P12 are treated with 20 λ of 3 *M* nitric acid which is free from chloride. The precipitate and the acid are carefully mixed while cold, and then heated on the steam bath while being continuously agitated. The formation of lumps of plastic sulfur will be avoided if these directions are followed. Heating on the steam bath is continued for 5 minutes. If a dark precipitate persists, which seems to dissolve slowly, 2 to 5 λ of 16 *M* nitric acid is added to the mixture, and heating is continued until solution appears to be complete. The mixture is centrifuged, and extract E21 is separated from residue R21. The residue is washed with three 5-λ portions of hot 1.2 *M* nitric acid, and the washings are combined with the extract.

**ESTIMATION OF THE QUANTITY OF MERCURY.** Residue R21 is treated with 5 λ of nitric-hydrochloric acid mixture which is freshly prepared by mixing equal volumes of the concentrated acids. During continuous agitation with a glass thread, the contents of the microcone are heated for 2 minutes at 40° C. The cone is then transferred to the steam bath and heated for 3 minutes while air is blown into the cone. Hereafter 0.05 ml 0.3 *M* hydrochloric acid and 0.01 ml 3 *M* ammo-

nium chloride are added; the mixture is agitated, briefly warmed on the steam bath, and centrifuged. The clear centrifugate is transferred to another cone, and the residue of sulfur is rejected without washing. The centrifugate is treated with hydrogen sulfide, and the quantity of mercury is estimated by comparing the volume of the mercuric sulfide precipitate with that of a like precipitate obtained from a known quantity of mercuric ion.

**CONFIRMATORY TESTS FOR MERCURY.** After removal of the supernatant solution, the precipitate of mercuric sulfide is agitated for 2 minutes at 40° C with 5  $\lambda$  of nitric-hydrochloric acid mixture, and then heated for 3 minutes on the steam bath while air is blown into the cone. The mixture is centrifuged.

**SLIDE TEST.** The clear centrifugate is taken up into a capillary pipet, and several small drops of it are deposited on a microscope slide. After evaporation to dryness, the reagent is added to the residues as described in Expt. 20.

**SPOT TEST.** The clear centrifugate is transferred to a watch glass and evaporated to dryness on a steam bath. The residue is dissolved in such a volume of 1 *M* nitric acid as to give an approximately 1 per cent solution of mercuric ion (1  $\lambda$  of the acid for each 10  $\gamma$  of mercury). The solution is used for the iodide test described in Expt. 11.

**ISOLATION OF METALLIC MERCURY.** The clear centrifugate is transferred to a microcone and treated with 0.1 ml saturated ammonium oxalate. After mixing, the contents of the microcone are briefly centrifuged to collect them in the point of the cone. One end of a bright, straight piece of copper wire of 0.1-mm diameter and 7-cm length is inserted in the solution, which is then heated for 1 minute on the steam bath. The wire is finally removed from the cone and rinsed with tap water. It is dried carefully by pressing between filter paper, and while being held over a piece of clean paper the amalgamated end is cut off. The isolation of the mercury by distillation in a capillary is performed as directed in Expt. 43.

**ISOLATION AND ESTIMATION OF SILVER.** Extract E21, which has been collected in the point of a cone by brief centrifuging, is heated on the steam bath and treated with exactly 1  $\lambda$  of 0.1 *M* ammonium chloride. The mixture is agitated, heated, and then examined for a precipitate or turbidity. After centrifuging, the point of the cone is inspected with a magnifying glass or under the microscope. A suitable microscope magnification is 20 to 40. Using the cell shown in Fig. 43, the point of the cone is immersed in water so as to obtain better optical conditions.

The absence of silver is proved if there is no precipitate or turbidity. If the solution is turbid, however, or contains a white precipitate, it is heated, agitated, and centrifuged until a clear supernatant solution is obtained. Then 1  $\lambda$  of 0.5 *M* ammonium chloride is added and mixed with the solution without disturbing the precipitate in the point of the cone. Three results are possible: (a) The supernatant solution remains clear. This indicates that the precipitation of silver chloride is complete. (b) When the reagent is added to the solution by centrifuging, it first forms a top layer in which the chloride-ion concentration is high. As a result, lead chloride crystallizes on the surface of the solution. On stirring, however, this precipitate dis-

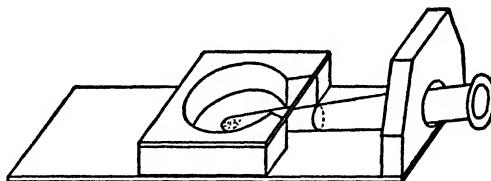


FIG. 43. Cell for observation of the contents of microcones.

solves, and the solution becomes clear, which indicates that the precipitation of silver chloride is complete. (c) The supernatant solution becomes turbid after mixing or a precipitate forms which is not distinctly crystalline. In this instance the whole contents of the cone are thoroughly mixed, heated, and centrifuged. The addition of 1- $\lambda$  portions of 0.5 *M* ammonium chloride is continued until the precipitation of the silver chloride is complete. The centrifugate S22 is finally removed from the precipitate P22 while still hot and transferred to a microcone.

The precipitate P22 of silver chloride is heated with 0.02 ml of 1.2 *M* nitric acid and centrifuged. The amount of silver is estimated from the volume of the precipitate. Heating, mixing, and centrifuging are repeated, and the wash liquid is removed while hot and combined with centrifugate S22.

#### CONFIRMATORY TESTS FOR SILVER.

**SLIDE TEST.** The silver chloride is washed once with 0.02 ml water. It is then treated with such a volume of concentrated ammonia as to give a 0.3 per cent solution of silver. After centrifuging, portions of the clear solution are transferred to slides and allowed to evaporate as described in Expt. 22.

**SPOT TEST.** Approximately 0.1 ml water and 0.01 ml 3 *M* ammonium chloride are added to the silver chloride, and the mixture is

treated with hydrogen sulfide until the conversion to silver sulfide is complete. The solution is removed and rejected. The sulfide precipitate is washed with three 0.02-ml portions of 0.12 *M* nitric acid and then dissolved by heating with 0.01 ml chloride-free 3 *M* nitric acid. The clear solution is transferred to a watch glass and evaporated to dryness on the steam bath. The residue is dissolved in a volume of distilled water to give an approximately 1 to 2 per cent solution of silver ion, which is used for the test described in Expt. 12.

**ISOLATION OF LEAD.** Centrifugate S22 is treated with 10  $\lambda$  of 6 *M* sulfuric acid. The microcone is first centrifuged and then placed on the steam bath. A stream of air is blown into the cone, and heating is continued until the volume of the solution has been reduced to a few lambdas. Then the microcone is transferred to a heating block, and, while a stream of air is blown into the cone, the temperature of the block is quickly raised until heavy white fumes emanate from the cone. The microcone is then removed from the heating block and thoroughly cooled by finally running tap water over its outside. While the microcone is held in a nearly horizontal position, 0.02 ml of water is deposited just below its rim. The water is first spread over the walls of the cone by means of a glass thread, and then it is allowed to flow into the point of the cone while its contents are thoroughly agitated. The microcone is briefly whirled in the centrifuge to collect its contents in the point. The contents of the point are mixed with the glass thread, and the mixture is allowed to stand for exactly 6 minutes. After this time the microcone is briefly centrifuged. If a precipitate P23 has formed, the clear supernatant solution S23 is immediately transferred to another cone. The precipitate of lead sulfate is washed with one portion of 5  $\lambda$  of 0.6 *M* sulfuric acid, and the washing is combined with centrifugate S23.

**ESTIMATION OF THE QUANTITY OF LEAD.** Precipitate P23 is treated with 5- $\lambda$  portions of 3 *M* ammonium acetate, with stirring after the addition of each portion of reagent, until the lead sulfate is completely dissolved. The solution is briefly heated on the steam bath, centrifuged, and separated from any gelatinous precipitate which may have formed. The clear solution is treated with an equal volume of 2 *M* acetic acid and then diluted with water to a volume of 0.05 ml. The cone is now placed on the steam bath, and its contents are treated with 5- $\lambda$  portions of 0.02 *M* potassium dichromate, with stirring after each addition, until the precipitation of the lead chromate is complete. The solution must finally show the orange color of dichromate ion. Heating on the steam bath and stirring are continued until the precipitate has become coarsely crystalline and settles rapidly. After

centrifuging, the amount of lead is estimated in the usual manner from the volume of lead chromate.

**CONFIRMATORY TESTS FOR LEAD.** The supernatant solution is removed from the lead chromate, and the precipitate is first washed with 0.01 ml of water and then dissolved by adding 3 *M* sodium hydroxide in 2-λ portions, with stirring after each addition, until a clear solution is obtained. Hydrogen sulfide is passed into the solution until precipitation is complete. The mixture is treated with twice its volume of water and centrifuged. The centrifugate is removed, and the precipitate is washed once with 0.01 ml of water. The lead sulfide is then dissolved in such a volume of 3 *M* nitric acid as to give an approximately 1 per cent solution of lead ion. The solution is freed from sulfur by centrifuging, and it is then ready for use in the *spot test* described in Expts. 11 and 13. For the *slide test*, the clear solution is mixed with twice its volume of copper test solution (10 mg Cu per ml). Several small portions of this mixture are evaporated just to dryness on a microscope slide. The residues are treated with nitrite reagent as directed in Expt. 23.

**ISOLATION OF BISMUTH AND DETECTION OF COPPER.** While air is blown into the cone, solution S23 is heated on the steam bath until it has been concentrated to one-half its original volume. The solution is then cooled by running tap water over the cone. While the solution is being cooled and agitated with a glass thread, concentrated ammonia is added in 5-λ portions until the mixture reacts alkaline toward litmus. The reaction of the mixture is tested by drawing the glass thread used in stirring across the edge of a piece of red litmus paper. A magnifying glass is desirable for observation of the color change. Finally, the alkaline mixture is treated with 5 λ more of the concentrated ammonia. The mixture is heated to almost boiling, and then the precipitate is collected by whirling in the centrifuge. The point of the cone is inspected for a precipitate of bismuth hydroxide by holding the cone in front of a black background and illuminating laterally. If a precipitate is not seen with the unaided eye, use of a magnifying glass or of the low-power microscope is suggested. Copper is indicated by a blue coloration of the supernatant solution. Strong lateral illumination and a black background being used for observation of the precipitate, the centrifugate S24 is transferred to another cone. The precipitate is washed with two 5-λ portions of approximately 1.2 *M* ammonia, and the washings are combined with the centrifugate.

**ESTIMATION OF THE QUANTITY OF BISMUTH.** The washed precipitate P24 is treated with 10 λ of freshly prepared stannite reagent (see

Expt. 26). After mixing and centrifuging, the quantity of bismuth is estimated from the volume of the black precipitate of metallic bismuth.

**CONFIRMATORY TESTS FOR BISMUTH.** After removal of the centrifugate, the black precipitate of metallic bismuth is washed with two 0.01-ml portions of hot water. It is then dissolved in  $5\lambda$  of 16 *M* nitric acid, and the solution is transferred to a microscope slide and evaporated just to dryness. The residue is dissolved in such a volume of 3 *M* nitric acid as to give an approximately 1 per cent solution of bismuth ion, which may be used for the slide tests described in Expts. 24, 25, and 26, or the spot test of Expt. 14.

**SEPARATION AND ESTIMATION OF COPPER.** The amount of copper may be estimated from the intensity of the blue coloration and the volume of centrifugate S24. It is obvious that standards for comparison may be prepared from copper test solution.

Centrifugate S24 is treated with  $1-\lambda$  portions of 6 *M* sulfuric acid until it reacts acid to litmus paper. The disappearance of the blue color of the tetramminocupric ion is an excellent internal indicator. Finally,  $2\lambda$  more of the sulfuric acid is added, and the acid solution is treated with 2 to 3 crystals of potassium iodide of approximately 1-mm edge ( $1-\lambda$  volume). The mixture is stirred until all the potassium iodide has dissolved, and then the liberated iodine is removed by heating on the steam bath and blowing air into the microcone. The mixture of cuprous iodide and solution is then treated with  $5\lambda$  of saturated ammonium thiocyanate. After the mixture has been agitated vigorously for several minutes, it is centrifuged, and the quantity of copper is estimated from the volume of precipitate. Then centrifugate S25 is transferred to another cone, and precipitate P25 is washed with two  $5-\lambda$  portions of cold water. The precipitate is reserved for later use. The washings are combined with the centrifugate, which is immediately treated as directed in the following paragraph.

**DETECTION AND ESTIMATION OF CADMIUM.** Centrifugate S25 is made alkaline by adding  $1-\lambda$  portions of 6 *M* ammonia, and then treated with  $2\lambda$  of 10 per cent potassium cyanide. The solution is centrifuged and separated from any precipitate which may have collected in the point of the cone. The clear solution is treated with a moderate current of hydrogen sulfide for 20 seconds. Separation of a yellow precipitate indicates the presence of cadmium. The mixture is treated with 0.02 ml of 6 *M* ammonia and more hydrogen sulfide. When the precipitation has become complete, the mixture is agitated vigorously and heated on the steam bath to cause flocculation of the

precipitate. After centrifuging, the quantity of cadmium is estimated from the volume of cadmium sulfide.

**CONFIRMATORY TESTS FOR CADMIUM.** After removal of the supernatant solution, the precipitate of cadmium sulfide is washed with two 0.01-ml portions 0.3 *M* ammonium chloride. The precipitate is then dissolved by heating with 0.01 ml of 6 *M* hydrochloric acid. The clear solution is transferred to a watch glass and evaporated to dryness on the steam bath. The residue is dissolved in a volume of water to give an approximately 0.1 per cent solution of cadmium ion, which is then ready for the slide test of Expt. 28 and the spot test of Expt. 16.

**CONFIRMATORY TESTS FOR COPPER.** Precipitate P25 is heated with 5  $\lambda$  of nitric-hydrochloric acid mixture (1 : 1). The solution is transferred to a microscope slide and evaporated to dryness. The residue is dissolved in a volume of water to give an approximately 0.1 per cent solution of copper ion which may serve for the slide test, Expt. 27, and the spot test, Expt. 15.

**COMPILED OF RESULTS.** Considering that 10  $\lambda$  of known solution III was taken for analysis, the concentrations of the various ions are calculated from the estimated amounts and listed as grams per liter. The results may be compared with the composition of the solution which is given in the Appendix.

## EXPERIMENT 51

### Analysis of an Unknown Solution Containing Ions of the Copper Group

Exactly 10  $\lambda$  of the unknown solution, which may contain 2 or 3 ions of the copper group, is taken for analysis. The procedure outlined in the preceding experiment is followed. The quantities are estimated as closely as possible, and the results are expressed as concentrations: gammas per lambda, milligrams per milliliter, or grams per liter.

For quick information on the scheme of analysis one may use Tables II and III. The washings are always combined with the centrifugates so as to render the procedure quantitative. The compounds which serve for estimation of quantities are indicated in the tables by bold-face type, and it may be seen at a glance when it is advisable to change to a straight-tip cone, provided that use of this type of cone is desired for estimations.

The proper choice between measuring pipet and calibrated capillary pipet is indicated throughout the directions by using the dimension milliliter if the former is preferable, and lambda if the latter is to be chosen.

At this point some advice may be desirable on preserving the substance under investigation when the analysis must be interrupted. As a rule, it is preferable to save solutions rather than precipitates. Sulfide precipitates oxidize if they are kept for some time, and many hydrated oxides become difficultly soluble if they dry out. Most solutions, however, may be kept in stoppered cones for considerable time. If undesirable oxidation is to be feared, as with sodium sulfide extracts, the solution is sealed into a capillary so that little air space is left. Residues obtained on evaporation of solutions may usually be kept for any length of time. Slides with such residues are placed under a glass bell jar. If the residue has been obtained on a watch glass, it is sufficient to place the watch glass with the concave side down on a clean piece of paper.

## EXPERIMENT 52

### Separation of the Metals of the Arsenic Group <sup>38</sup>

Exactly  $10\lambda$  of known solution IV is transferred to a microcone by means of a centrifugal pipet, and the precipitation with hydrogen sulfide is carried out exactly as described in Expt. 49. Centrifugate S11 is tested for completeness of precipitation as outlined.

**SEPARATION OF THE ARSENIC AND COPPER GROUPS.** Precipitate P11 or the combined precipitate P11 and P12 is treated with  $15\lambda$  of sodium sulfide-hydroxide reagent. The mixture is heated on the steam bath for 3 minutes, with stirring, any residue being disintegrated. Then  $15\lambda$  of  $0.6\text{ M}$  sodium hydroxide is added, and after mixing, residue R13 and extract E13 are separated. The residue is heated for 2 minutes with  $5\lambda$  sodium sulfide-hydroxide reagent. The mixture is diluted with 0.01 ml of water and agitated. After centrifuging, the second extract is transferred to E13. The extraction with  $5\lambda$  of reagent and 0.01 ml of water is repeated once.

The combined extracts E13 are treated with 0.03 ml of  $6\text{ M}$  ammonium acetate for the precipitation of mercuric sulfide. The mixture is agitated and then heated for 1 minute on the steam bath. After centrifuging, the supernatant solution S14 is transferred to another microcone. Precipitate P14 is washed with two 0.01-ml portions of approximately  $0.5\text{ M}$  ammonium sulfide. The washings are com-

bined with centrifugate S14. Finally, precipitate P14 is made into a slurry with some 0.5 *M* ammonium sulfide solution and transferred to residue R13. The combined solids R13 and P14 contain the elements of the copper group, and they are now ready for treatment with 3 *M* nitric acid as outlined in the preceding experiment. This time, the analysis of the copper group may be omitted.

**PRECIPITATION OF THE SULFIDES OF THE ARSENIC GROUP.** Centrifugate S14 is treated with 0.08 ml of 2 *M* *sulfuric acid*. The acid is added in small portions, with stirring after each addition. The mixture is heated to approximately 80° C and agitated until flocculation takes place. After centrifuging, the acid reaction of the supernatant solution is confirmed by transferring a trace of it to litmus paper. The centrifugate S31 is then removed and rejected. Precipitate P31 of the sulfides of the arsenic group need not be washed, but the centrifugate should be removed as completely as possible to prevent dilution of the hydrochloric acid in the following operation.

**SOLUTION OF THE SULFIDES OF THE ARSENIC GROUP.** Precipitate P31 is treated with 20  $\lambda$  of 12 *M* hydrochloric acid. The microcone is placed into a water bath of 80° C, and the mixture in the cone is continuously agitated with a glass thread until the action of the acid has come to a stop. Any sulfide which remains undissolved is now brought into solution by adding potassium bromate. Particles of the solid salt of 0.1- $\lambda$  volume (0.6-mm diameter) are added one at a time. The salt is spread on a glass plate, and the selected particles are picked up with the moist glass thread used in stirring. The bromate must be added sparingly, and a new portion is added only after the action of the preceding addition is exhausted. The treatment with bromate and agitating of the mixture are continued until all sulfide has dissolved and the solution permanently assumes the orange color produced by dissolved bromine. The residue of sulfur is centrifuged into the point of the microcone, and solution S32 is transferred to a clean, dry pressure cone, Fig. 11. The residue R32 of sulfur is washed with two 5- $\lambda$  portions of 12 *M* hydrochloric acid. The washings are combined with solution S32, and residue R32 is rejected.

**REDUCTION WITH PHOSPHOROUS ACID, HEATING IN A SEALED TUBE.** The contents of the pressure cone are treated with 14  $\lambda$  of 3 *M* phosphorous acid and 1  $\lambda$  of 9 *M* hydrobromic acid. They are then collected in the point of the cone by means of the centrifuge. The pressure cone is drawn out at  $\alpha$  to a short capillary and sealed. Its contents are mixed by holding the top of the cone lightly with one hand while repeatedly striking with the index finger of the other at the tip of the cone. The pressure cone is submerged in water of 90° C until

the color of bromine has disappeared. Then the cone is taken from the bath and cooled with tap water. The cone is violently shaken to wet its interior walls. It is placed into the centrifuge, whirled briefly to collect the liquid in the point, and cut open at *a*.

**ISOLATION OF ARSENIC BY DISTILLATION OF THE TRICHLORIDE.** The distilling flask proper, Fig. 44, is made of 6-mm-bore Pyrex tubing, and it has near the bottom a graduation mark indicating a volume of 15  $\lambda$ . It is only 30 to 40 mm high, and the side arm, formed by a

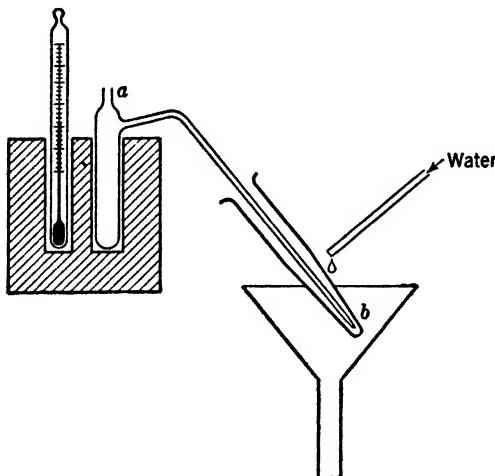


FIG. 44. Distillation of arsenic trichloride on the milligram scale, approximately half natural size.

capillary of 0.5- to 1-mm bore, is set on as close to the top as possible. The short tube *a* has a bore of approximately 2 mm so that a capillary pipet may be inserted with ease.

By means of a capillary pipet the solution is transferred from the pressure cone to the distilling flask, which must be either dry or is to be rinsed with concentrated hydrochloric acid before use. The pressure cone is rinsed with 5  $\lambda$  of concentrated hydrochloric acid, and the rinsings too are transferred to the distilling flask. The flask is then placed in a metal heating block which accommodates the whole length of the flask up to the side arm. Tube *a* is connected with a thin rubber tubing to a supply of clean air which is dried by passing it through a tube with Anhydrene or calcium chloride. The rate of air flow is regulated by pushing into the rubber tubing a wad of cotton and applying in the same place a screw clamp to the rubber tubing. With the screw clamp closed, the side arm *b* is inserted in a micro-cone containing 0.04 ml of water. Ice-cooled water is made to run

over the outside of the cone. The stream of air is regulated so that not more than 2 bubbles per second appear in the microcone. The block is heated to 130° C and held at this temperature. The distillation is continued until the volume of the solution in the distilling flask is between 10 and 15  $\lambda$ . If the heating block is not fitted with windows for observation, it becomes necessary to withdraw the distilling flask from time to time from the block in order to observe the progress of the distillation. When finally only 10 to 15  $\lambda$  of the liquid is left in the distilling flask, the flask is removed from the heating block and the side arm capillary is withdrawn from the microcone. The rubber tubing is removed from tube *a*, and 10  $\lambda$  of 12 *M* hydrochloric acid is added to the residue in the distilling flask. The distillation is repeated until the volume of the residue has again shrunk to 10 or 15  $\lambda$ . The microcone containing the first portion of the distillate is used for the collection of the second portion also. The distillation is broken off as already described. The outside of the delivery tube is rinsed with 0.01 ml of water before the stream of air is shut off.

**DETECTION AND ESTIMATION OF ARSENIC.** The distillate is treated with 0.04 ml of 12 *M* hydrochloric acid and then diluted with water to a volume of approximately 0.1 ml. The volume may be easily estimated by comparison with a microcone of like dimensions containing 0.1 ml of water. The solution is saturated with hydrogen sulfide at room temperature, and then the cone is closed with a pressure cap, Fig. 10, and heated on the steam bath. The treatment with hydrogen sulfide is repeated until the precipitation of arsenic sulfide is complete. The precipitate is then collected by means of the centrifuge, and the quantity of arsenic is estimated from the volume of the precipitate.

**CONFIRMATORY TESTS FOR ARSENIC.** The supernatant solution is removed from the precipitate of arsenic trisulfide, and the precipitate is washed once with approximately 0.03 ml of water. The sulfide is then treated with 5  $\lambda$  of nitric-hydrochloric acid mixture (1 : 1). The microcone is placed in a water bath of approximately 60° C, and the mixture is agitated until the residue is colorless. After centrifuging, the clear solution is transferred to a watch glass and evaporated to dryness on the steam bath.

**BETTENDORFF'S TEST.** The residue is dissolved in 10  $\lambda$  of 12 *M* hydrochloric acid. The solution is taken up into a capillary which contains 3  $\lambda$  of stannous chloride reagent, and the directions given in Expt. 44 are followed.

**SLIDE TEST AND SPOT TEST.** The residue on the watch glass is dissolved in a volume of 2 *M* nitric acid to give an approximately 1 per

cent solution of arsenic. The solution may be used for either slide test, Expt. 29, or spot test, Expt. 17.

**ISOLATION AND ESTIMATION OF ANTIMONY.** The residue in the distilling flask is transferred to a microcone by means of a capillary pipet. The flask is rinsed with two 25- $\lambda$  portions of a solution obtained by adding 20 ml of 3 *M* sulfuric acid and 5 ml of 6 *M* hydrochloric acid to 25 ml of water. The rinsings are combined with the residue of the distillation. Finally, 2  $\lambda$  of 3 *M* phosphorous acid and 10  $\lambda$  of 3 *M* hydrochloric acid are added to the solution in the microcone. The cone is then placed into a large beaker filled with water of 75° C, and a slow stream of hydrogen sulfide is bubbled through the solution for 10 minutes. Separation of an orange or grayish black precipitate indicates the presence of antimony. By means of the centrifuge, the precipitate is collected in the point of the cone, and the amount of antimony is estimated from the volume of antimony trisulfide. Centrifugate S34 is transferred to another cone. The sulfide precipitate P34 is washed with two 5- $\lambda$  portions of hot 1.2 *M* hydrochloric acid, and the washings are combined with S34.

**CONFIRMATORY TESTS FOR ANTIMONY.** The antimony trisulfide is treated with a volume of 10 *M* hydrochloric acid to give an approximately 0.5 per cent solution of the metal. The mixture is heated in a water bath at 60° to 70° C for 5 minutes with continuous stirring. After centrifuging, the clear solution may be used for the slide test of Expt. 24 or the spot test of Expt. 18.

**DETECTION AND ESTIMATION OF TIN.** The microcone containing solution S34 is placed in cold water, and its contents are made alkaline by adding 5- $\lambda$  portions of concentrated ammonia; after each addition the contents are stirred and the reaction is tested by transferring a trace of the solution to red litmus paper by means of the glass thread. Approximately 30  $\lambda$  of 15 *M* ammonia will be required; a large excess of ammonia must be avoided.

The alkaline solution is saturated with hydrogen sulfide, and a slow current of the gas is then passed through the solution for 2 more minutes. The solution is then heated on the steam bath and treated with 2- $\lambda$  portions of 3 *M* sulfuric acid, with stirring and testing after each addition, until the mixture reacts acid toward litmus paper. Then 2  $\lambda$  more of the acid is added, the cone is taken from the steam bath, and the mixture is saturated with hydrogen sulfide while it cools to room temperature. The opening of the cone is closed with a pressure cap, and the sealed cone is placed in boiling water for 5 minutes. Separation of a yellowish precipitate indicates the presence of tin.

The precipitate of stannic sulfide is collected in the point of the cone by means of the centrifuge, and the quantity of tin is estimated from the volume of the precipitate.

**CONFIRMATORY TESTS FOR TIN.** The supernatant solution is removed from the precipitate of stannic sulfide, and the precipitate is washed with a 0.02-ml portion of 1 *M* ammonium chloride. A volume of 6 *M* hydrochloric acid is added to give an approximately 0.2 per cent solution of stannic ion. The mixture is heated on the steam bath until solution is complete and the hydrogen sulfide is expelled. After centrifuging, the solution is separated from any residue and taken up into a capillary pipet.

**SLIDE TEST.** Approximately 2  $\lambda$  of the solution is transferred to the center of a microscope slide, which is then inverted and placed on the opening of a chlorine water bottle so that the hanging drop of tin solution is exposed to chlorine vapor. The slide is taken off the bottle after 2 minutes, and the test drop is treated with rubidium chloride as outlined in Expt. 31.

**SPOT TEST.** The tin solution is transferred from the capillary pipet to a microcone, and a rectangular piece of magnesium ribbon (0.5 mm by 5 mm) is added. When the magnesium has dissolved, the solution is immediately taken back into the capillary pipet and used as directed in Expt. 19.

**COMPILED OF RESULTS.** Considering that 10  $\lambda$  of known solution IV was taken for analysis, the concentrations of arsenic, antimony, and tin are calculated from the estimated amounts and tabulated as grams per liter. The results may be compared with the composition of the solution listed in the Appendix.

### EXPERIMENT 53

#### Analysis of an Unknown Solution Containing Ions of the Copper and Arsenic Groups

Exactly 10  $\lambda$  of the unknown solution, which may contain 3 or 4 ions, is taken for analysis. The procedure outlined for analysis of known solution IV is followed. The quantities are estimated as closely as possible, and the concentrations found are expressed as grams per liter. To facilitate quick orientation, a tabular outline of the scheme of analysis is given on the following pages. The compounds serving for estimation of the quantities are emphasized by bold-face type.

TABLE II

## PRECIPITATION OF THE HYDROGEN SULFIDE GROUP AND SEPARATION OF THE COPPER AND ARSENIC GROUPS

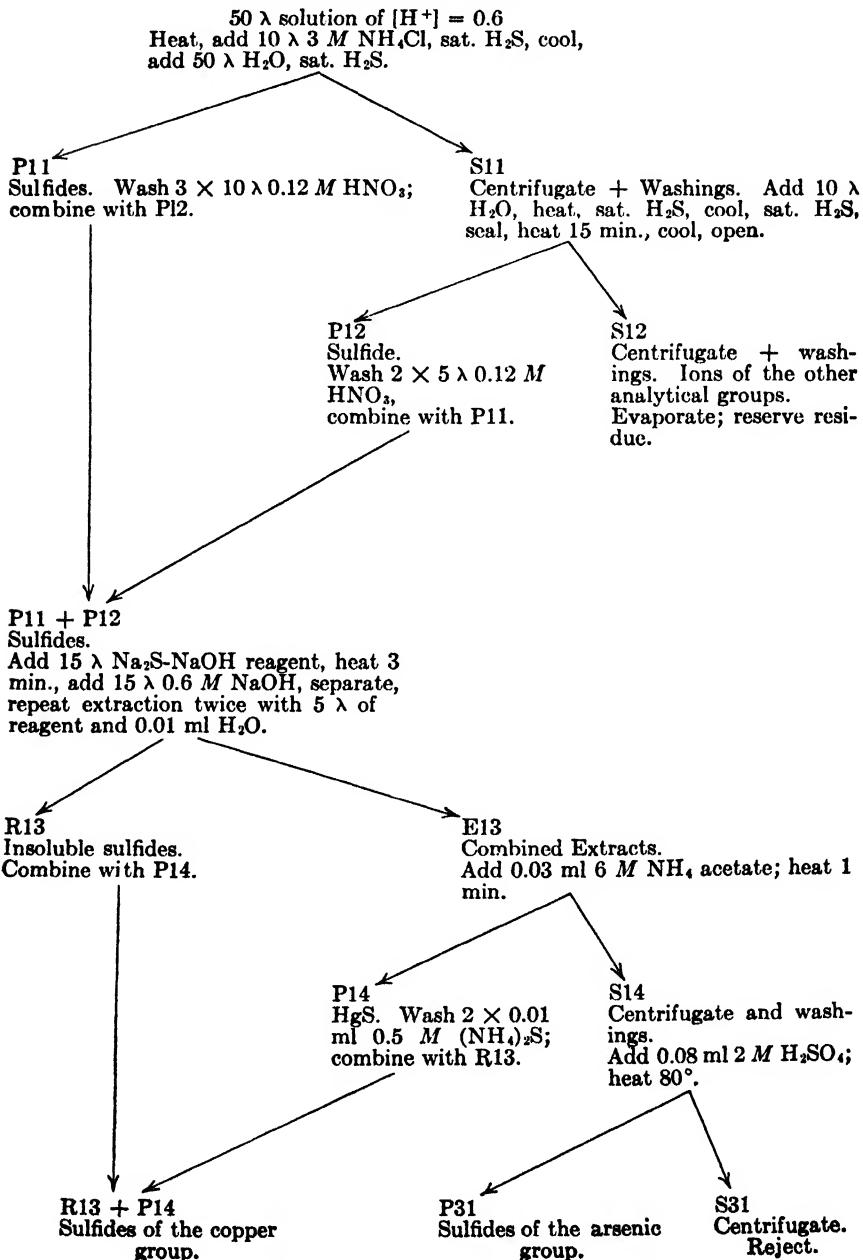


TABLE III  
ANALYSIS OF THE COPPER GROUP

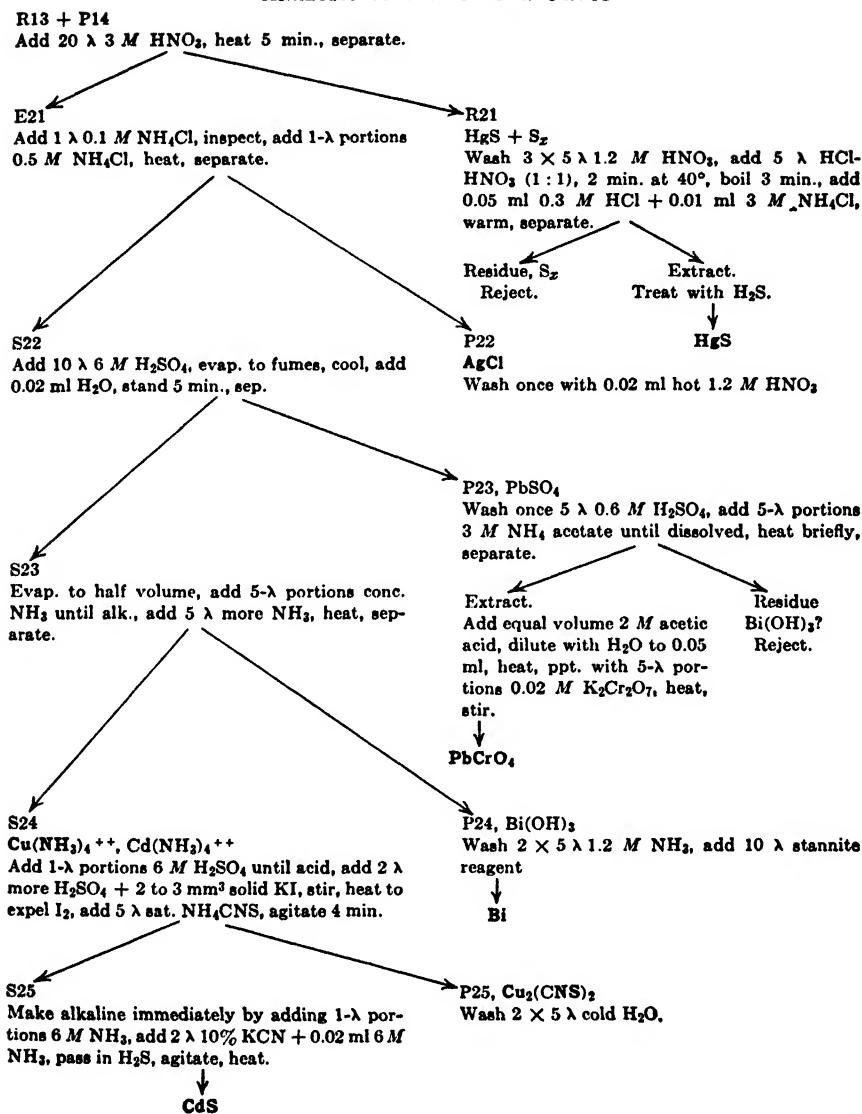


TABLE IV

## ANALYSIS OF THE ARSENIC GROUP

P31

Remove centrifugate as completely as possible, add 20  $\lambda$  12 M HCl, agitate, heat at 80°, add 0.1-mm<sup>3</sup> portions of solid KBrO<sub>3</sub> until sulfides are dissolved and solution is orange, separate.

S32

Add 14  $\lambda$  3 M H<sub>3</sub>PO<sub>3</sub> + 1  $\lambda$  9 M HBr, seal, mix, heat at 90° until colorless, cool, mix, open, transfer to distilling flask, rinse with 5  $\lambda$  12 M HCl, distil into 0.04 ml H<sub>2</sub>O until 10 to 15  $\lambda$  is left in flask, add 10  $\lambda$  12 M HCl, again distil to 10 to 15  $\lambda$  residue, rinse side arm capillary with 0.01 ml H<sub>2</sub>O.

R32, S<sub>x</sub>

Wash 2  $\times$  5  $\lambda$  12 M HCl, then reject.

R33

Residue of distillation.

Transfer to microcone, rinse 2  $\times$  25  $\lambda$  mixture (20 ml 3 M H<sub>2</sub>SO<sub>4</sub> + 5 ml 6 M HCl + 25 ml H<sub>2</sub>O), add 2  $\lambda$  3 M H<sub>3</sub>PO<sub>3</sub> + 10  $\lambda$  3 M HCl, place in water of 75° and pass in H<sub>2</sub>S for 10 min., separate.

D33

Distillate.

Add 0.04 ml 12 M HCl, dilute with H<sub>2</sub>O to 0.1 ml, sat. with H<sub>2</sub>S, seal, heat in steam, repeat H<sub>2</sub>S treatment until pptn. complete.

↓  
As<sub>2</sub>S<sub>3</sub>

S34

Cool with water, make alkaline by adding conc. NH<sub>3</sub> in 5- $\lambda$  portions, sat. with H<sub>2</sub>S, then heat and acidulate with 2- $\lambda$  portions 3 M H<sub>2</sub>SO<sub>4</sub>, add 2  $\lambda$  more of 3 M H<sub>2</sub>SO<sub>4</sub>, sat. with H<sub>2</sub>S while cooling to room temp., seal, place in boiling water for 5 min., centrifuge.

P34, Sb<sub>2</sub>S<sub>3</sub>

Wash 2  $\times$  5  $\lambda$  hot 1.2 M HCl.

↓  
SnS<sub>2</sub>

EXTRACTION OF AQUEOUS SOLUTIONS WITH ORGANIC SOLVENTS<sup>29</sup>

Small volumes of liquids may be extracted without the use of any special apparatus. If one treatment with the solvent is sufficient, the solution and the solvent are taken up into a capillary pipet which is

<sup>29</sup> F. Emich, *Mikrochemisches Praktikum*, Munich, J. F. Bergmann, 1924, p. 142. *Umsetzungen sehr kleiner Stoffmengen* in A. Stähler, E. Tiede, and F. Richter, *Handbuch der Arbeitsmethoden in der anorganischen Chemie*, 2nd vol., Berlin, W. de Gruyter, 1925, p. 846.

then sealed at both ends. The contents of the tube are then repeatedly centrifuged from one end of the capillary to the other. Finally the capillary is cut at the interface of the two liquids. When working with solvents of high vapor tension, it is necessary to open the gas space first. If the extraction must be repeated, it is preferable to work in a straight-tip cone as outlined in the following experiment. When using solvents of low boiling point, it is advisable to cool the cone by immersing it most of the time in ice water.

#### EXPERIMENT 54

##### Extraction of Ferric Chloride

One volume of diethylether is shaken with 2 volumes of 6 *M* hydrochloric acid. The mixture is kept in an amber bottle.

Approximately 0.01 ml of ferric chloride test solution (10 mg Fe per ml) is transferred to a watch glass. The solution is treated with approximately 2  $\lambda$  of aqua regia and evaporated to dryness on the steam bath. The residue is moistened with 5  $\lambda$  of concentrated hydrochloric acid, and evaporation to dryness is repeated. The watch glass is allowed to cool to room temperature. The residue is dissolved in 0.01 ml 6 *M* hydrochloric acid, and the solution is transferred to a straight-tip cone having a tip of 1-mm bore. The cone is immersed in ice water, and, when it has become thoroughly cooled, 0.02 ml of the diethylether is added and the cone is briefly centrifuged to collect its contents in the capillary part. The cone is immediately returned to the cooling bath, and its contents are thoroughly mixed by means of a glass thread with a bead on its end. A suction device as shown in Fig. 15C is made ready, and a microcone is placed in the suction tube to serve as receiver. The straight-tip cone is removed from the cooling bath, briefly centrifuged, and returned to the bath for a few seconds. With slight suction, the ether layer is siphoned off into the microcone, the suction being interrupted immediately when the transfer is complete so as not to promote the evaporation of the ether from the microcone. The extraction is repeated twice. Then the microcone is quickly removed from the suction tube and whirled in the centrifuge. The ether is evaporated by blowing a stream of air into the microcone, and the residue is dissolved in 0.01 ml 6 *M* hydrochloric acid. The solution is treated with 0.01 ml 1 *M* potassium thiocyanate, mixed, and centrifuged. The residue from the extraction is transferred from the straight-tip cone to a microcone, and it is likewise

treated with 0.01 ml 1 *M* potassium thiocyanate. The colorations obtained with extract and residue are compared.<sup>40</sup>

#### TREATMENT OF INSOLUBLE SUBSTANCES<sup>41</sup>

In the practice of analytical chemistry the most important treatments for the attack of insoluble substances are fuming with hydrofluoric acid and fusions with sodium carbonate, sodium peroxide, and potassium pyrosulfate. The technique employed must permit efficient quantitative transference of the material under investigation before and after treatment, and for this reason, it is advisable to forego the use of small crucibles or of platinum sheet in milligram procedures. The following techniques are suggested.

**FUMING WITH HYDROFLUORIC ACID.** By means of an oxygen flame a sphere of 2-mm diameter is fused at the end of a platinum wire of 0.5-mm diameter. The insoluble substance is made into a slurry with dilute sulfuric acid or with water. The slurry is taken up into a capillary and transferred to the platinum bead by adding small portions at a time, and evaporating after each addition. The platinum bead is not heated directly, but a microflame is applied to the wire at a distance of several millimeters from the bead. When the transfer has become complete, the residue on the bead is treated with hydrofluoric acid or hydrofluoric-sulfuric acid mixture, a drop of which is held ready on a platinum sheet. The acid is added by means of a platinum loop to a part of the platinum bead which holds little or none of the substance. The acid is slowly evaporated by heating the wire at a distance from the bead, the treatment being repeated as often as appears necessary. For the extraction of the residue the platinum wire is placed with the bead down inside a microcone containing the solvent.

**SODIUM CARBONATE FUSION.** A straight platinum wire of 0.5-mm diameter is used. Some anhydrous sodium carbonate is placed on a piece of platinum foil, and small portions of it are taken up by touching them with the end of the heated platinum wire until a carbonate bead of the desired size is obtained. A bead of 1.5-mm diameter at the "equator" contains approximately 8 mg of sodium carbonate.

<sup>40</sup> On the shortcomings of this separation one is referred to S. E. Q. Ashley and W. M. Murray, *Ind. Eng. Chem., Anal. Ed.*, 10, 367 (1938).

<sup>41</sup> A. A. Benedetti-Pichler, paper presented before the Microchemical Section, at the 96th Meeting of the American Chemical Society, Milwaukee, Wis., Sept. 5 to 9, 1938. A somewhat different technique was described earlier by Ch. van Brunt, *Ind. Eng. Chem., Anal. Ed.*, 10, 224 (1938).

The exact amount of flux may be determined by weighing the wire with and without bead, or a weighed amount of sodium carbonate may be placed on platinum foil and completely taken up into the bead. The bead is continuously kept at the end of the wire by heating the wire with a microflame close to the near side of the bead. If the use of illuminating gas is objectionable, a flame of purified hydrogen may serve, or an electrically heated wire, stretched horizontally, may take the place of the flame. The material under investigation is made into a slurry with water and transferred to the bead in small portions, evaporating and fusing after each addition. If the substance is weighed on a sheet of platinum foil, which should have a high polish, it can be quantitatively mopped up with the molten bead. In all instances the mixture is finally fused until gas bubbles no longer appear in the bead. This requires hardly more than 1 minute. To dissolve the melt, the wire is placed in a microcone with the bead down. Obviously, it is preferable not to fuse the wire into a glass handle, but to hold it with forceps which can be locked after insertion of the wire.

**SODIUM PEROXIDE FUSION.** The fusion is carried out on a straight nickel wire of 0.5-mm diameter. One pellet of sodium hydroxide is fused on a sheet of nickel, and a bead is collected on the end of the nickel wire by dipping it repeatedly into melt, allowing the salt on the wire to solidify during the intervals. The material under investigation is added as a slurry in water, or it is mopped up from a platinum or nickel sheet with the cold sodium hydroxide bead which, to this end, is exposed briefly to air so that its surface becomes moist. After fusing briefly, sodium peroxide is taken up with the bead in a similar manner, and the bead is fused again. Addition of peroxide and fusion may be repeated once or twice.

**PYROSULFATE FUSION.** The fusion is carried out on a straight platinum wire of 0.5-mm diameter. A thick paste is prepared of powdered potassium sulfate and concentrated sulfuric acid. The paste is taken up with the end of the wire, and a bead of proper size is fused there and then heated until fumes of sulfur trioxide start to be given off. The material under investigation is added as a slurry in concentrated sulfuric acid. The fusion is carried out by heating the wire behind the bead slowly in the edge of the lowest portion of the Bunsen flame. The bead must be from 20 to 10 mm outside the flame.

## EXPERIMENT 55

**Analysis of an Alloy Containing Metals of the Copper and Arsenic Groups**

Gas pipe, 15 cm long, sealed at one end.

The alloy is supplied either as filings or in the form of fine wire. Approximately 0.3 to 0.5 mg of it is weighed on the analytical balance. The metal is transferred to a microcone and treated with 5  $\lambda$  of 16 *M* nitric acid which is added in portions of 1  $\lambda$ . When the reaction is complete, the mixture is evaporated to dryness on the steam bath. The residue is warmed briefly with 10  $\lambda$  of 3 *M* nitric acid. Any insoluble white residue of stannic and antimonic oxides is collected in the point of the cone by means of the centrifuge. The clear solution is transferred to another microcone, and the residue is washed with two 0.01-ml portions of water. The washings are combined with the clear solution which is then treated with hydrogen sulfide as described in Expt. 49.

The residue of stannic and antimonic oxides is made into a slurry with water and transferred to a pressure cone, preferably of Pyrex glass. The water is removed after the solid has collected in the point of the pressure cone, and the oxide mixture is treated with 10  $\lambda$  of sodium sulfide-hydroxide reagent. After centrifuging, solid and liquid are carefully mixed. The pressure cone is sealed, and then heated in a bath of isoamyl alcohol, b.p. 130° C. A short piece of gas pipe<sup>9</sup> is closed at one end. Approximately 5 ml of amyl alcohol and the pressure cone are introduced. The open end of the pipe is closed with a cork, through which a glass tubing 20 cm long is passed, which serves as reflux condenser. The gas pipe is clamped into a universal stand which is best placed under the hood. The amyl alcohol is heated so that a more or less stationary ring of condensate holds its position for 15 minutes in the lower part of the glass tube just above the cork. Then the flame is removed, and the apparatus is allowed to cool to room temperature. The amyl alcohol is saved; the pressure cone is removed from the gas pipe, centrifuged, and reserved. The solution contains the elements of the arsenic group, and it may later be combined with S13 or S14. The precipitate contains metals of the copper group, and it may be combined with R13.

An attempt should be made to estimate the quantities of the various metals as accurately as possible. When the analysis is completely finished, and not before, the results of the estimations are to be cal-

culated and expressed in per cent by weight. The findings should permit assignment of the proper name to the alloy.

### SUGGESTIONS FOR ADDITIONAL EXPERIMENTS

It is understood that any tried macro scheme may be applied to the analysis of small samples. For serious work, the scheme of A. A. Noyes and Bray<sup>42</sup> is recommended. The qualitative analysis of most of the groups of that scheme has been performed on a small scale,<sup>43</sup> and reports on similar work concerning the rest may be expected to follow in a short time. The schemes of A. A. Noyes<sup>44</sup> for the analysis of the chloride and sulfate groups of anions have been tested by L. Branconi and K. D. Fleischer.<sup>45</sup> No difficulties were encountered.

The analysis of the glaze of pottery offers an interesting example for practice. A piece of broken pottery approximately 2 cm square is split through the middle by means of chisel and hammer so that only one side of each half has glaze coating. A large block of paraffin is placed in a tray, and by means of a pilot flame an area in the center of the top surface of the paraffin block is heated until a small pool of molten paraffin is formed. One fragment of the piece of pottery is placed, with the glazed surface down, in the molten paraffin, which is then allowed to solidify. While running tap water over the specimen,

<sup>42</sup> A. A. Noyes and W. C. Bray, *A System of Qualitative Analysis for the Rare Elements*, New York, Macmillan, 1927.

<sup>43</sup> Selenium Group: A. A. Benedetti-Pichler and J. R. Rachele, *Ind. Eng. Chem., Anal. Ed.*, 9, 589 (1937).

Osmium and ruthenium: A. A. Benedetti-Pichler and J. R. Rachele, *Mikrochemie*, 24, 16 (1938).

Tantalum, tungsten, and gold groups, centigram procedure: Christina C. Miller and A. J. Lowe, *J. Chem. Soc.*, 1940, 1258, 1263.

Thallium group: A. A. Benedetti-Pichler and W. F. Spikes, *Mikrochemie*, 19, 239 (1936).

Tellurium and copper groups: B. S. Alstdott and A. A. Benedetti-Pichler, *Ind. Eng. Chem., Anal. Ed.*, 11, 294 (1939).

Aluminum-chromium group, gallium, iron, manganese, zinc, nickel, and cobalt: A. A. Benedetti-Pichler and W. F. Spikes, *Mikrochemie*, Molisch-Festschrift, p. 3, 1936.

Alkaline-earths group: A. A. Benedetti-Pichler, Wm. R. Crowell, and Cl. Donahoe, *Ind. Eng. Chem., Anal. Ed.*, 11, 117 (1939).

Alkali group: A. A. Benedetti-Pichler and J. T. Bryant, *ibid.*, 10, 107 (1938).

An investigation of the analysis of platinum metals has been carried out by W. F. Whitmore and H. Schneider, *Mikrochemie*, 17, 279 (1935).

<sup>44</sup> A. A. Noyes, *Qualitative Chemical Analysis*, 9th ed., New York, Macmillan, 1922.

<sup>45</sup> Masters' Theses, New York University, 1940.

the clay is removed by means of a dental drill so that nothing but the glaze is finally left imbedded in the paraffin. The glaze is removed by means of a knife and forceps. It is placed between pieces of filter paper on a heating block and weighted down with a piece of metal. On heating to approximately 80° C most of the adhering paraffin is absorbed by the paper. The glaze is transferred onto a stack of 3 to 4 disks of filter paper and washed with several drops of ether, which are added one at a time from a pipet. After pressing between filter paper and drying in the oven, the glaze is crushed in a clean agate mortar and ground to a fine powder. Approximately 1 mg of the powder is weighed off on a small piece of platinum foil, from which it is later collected into a carbonate bead of approximately 8 mg sodium carbonate. After fusion, any one of the customary schemes of analysis may be followed. It is advisable to save the rest of the powdered glaze for repetition of the analysis.

### GAMMA PROCEDURES

#### CHEMICAL WORK IN CAPILLARY CONES

The operations of qualitative analysis are performed in a tiny centrifuge cone of 1-λ capacity. The technique is essentially the same as in working with the microcone or centrifuge cone, and any reliable scheme of analysis may be followed. A scheme closely similar to that outlined in Tables II, III, and IV has been successfully applied by Cefola<sup>46</sup> to the separation of the common elements of the hydrogen sulfide group. Of course, one thousand times smaller masses and volumes of sample, reagents, and solutions were taken than indicated in the above-mentioned tables for the milligram procedure.

Because of the small volumes which must be handled, it is necessary to perform all operations which deal with the measuring or transfer of matter with the aid of mechanical devices while observing through a low-power microscope. Furthermore, solutions of considerably less than 1-λ volume evaporate in a very short time when exposed to air which is not saturated with water vapor. Thus, most of the work must be performed inside a moist chamber mounted on the stage of the microscope. Small volumes of reagents and wash liquids are held ready for use within this chamber. A mechanically operated micropipet, which is inserted through one side of the chamber, serves for the transfer of liquids.

<sup>46</sup> Michael Cefola, Ph.D. Thesis, New York University, 1941.

Apparatus and technique are essentially simple. Because of the mechanical and optical aids, all operations can be performed with ease and confidence. By projecting the microscopic images on a screen, Dr. Cefola has repeatedly demonstrated the experiments, which are described below, to audiences<sup>47</sup> of sixty to several hundred people. The practical value of a technique which permits qualitative analysis of specimens of 0.1- to 1-γ mass and the approximate estimation of the quantities of the constituents found is beyond question.

### APPARATUS

**Microscope.** The stand must be equipped with a built-in revolving and centerable mechanical stage without excessive superstructure and with backward and forward mechanical motions permitting maximum displacements of 6 to 9 cm. One micrometer eyepiece having a magnification of 5 will suffice. A revolving nosepiece permits rapid interchange of objectives. A nosepiece which allows separate centration of each objective is desirable but not necessary. Objectives of the magnifications 5, 10, and 20 are needed. The eyepiece micrometer is to be calibrated for use with these objectives. A bright-field condenser, which can be focused on a plane 10 mm above the stage, is desirable. Stands of the type described are used by biologists for micromanipulation under high magnification, and they are usually equipped with a rack-and-pinion device for focusing and a micrometer screw for fine adjustment of the focus, which, however, is rarely needed when working in the capillary cone.

**Manipulator.** Mechanical manipulators of the kind shown in Fig. 45M are made by several optical firms. Three smooth rack-and-pinion motions, each permitting a displacement of approximately 10 cm, produce the desirable flexibility of movement. In addition, rotation around the vertical column as axis is usually made possible. The shank of the micropipet *p* is inserted in the pipet holder *h*, which, in turn, is held in the clamp of the manipulator.

**Base.** Microscope and manipulator are placed side by side upon a wooden board and secured in their correct positions by means of small wooden blocks as indicated in Fig. 45. The proper distance between manipulator and microscope is determined as follows. The top

<sup>47</sup> Metropolitan Microchemical Society on Jan. 11, 1940, at the Washington Square College; New York Section of the American Chemical Society on April 5, 1940, at the Hotel Pennsylvania; Division of Laboratories, In-Service Training Course of the City of New York, Department of Hospitals, on Dec. 20, 1940, at the Washington Square College.

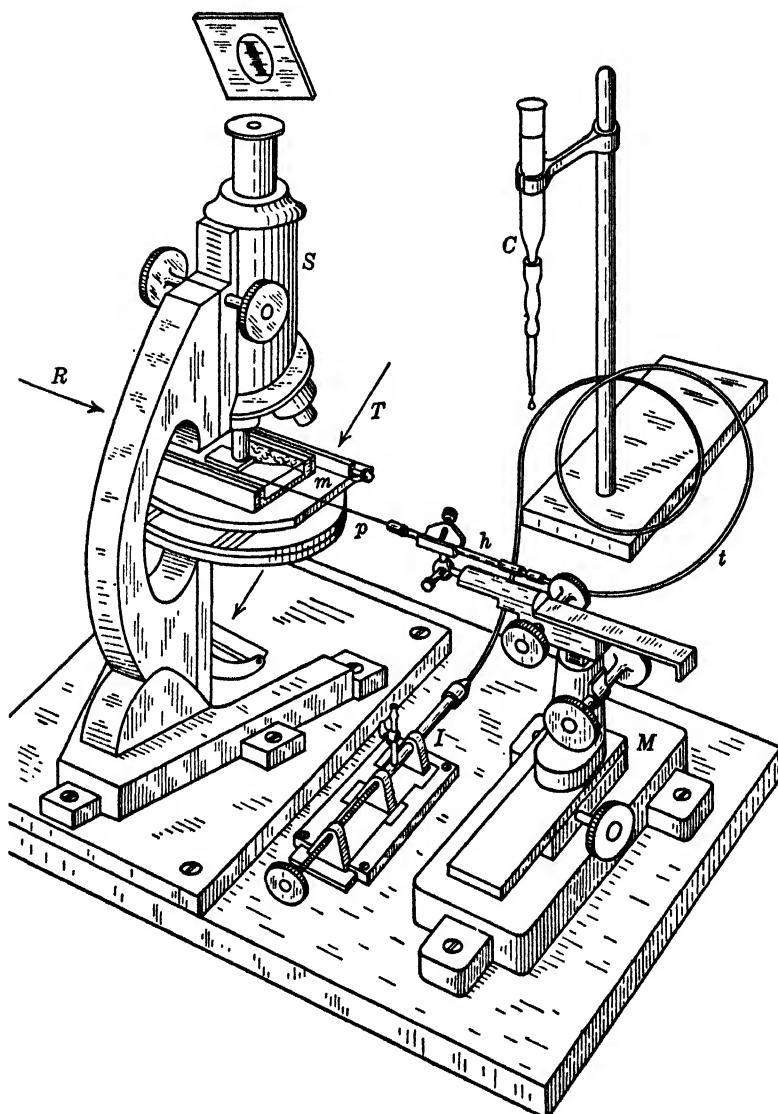


FIG. 45. Working in the capillary cone. Microscope with mechanical stage *m* and moist chamber; manipulator *M*; plunger device *I* connected by means of copper tubing *t* with pipet holder *h* and micropipet *p*; container *C* for distilled water used in rinsing micropipets.

pinion of the manipulator is operated to advance the clamp of the manipulator all the way toward the microscope. The two other pinions should engage approximately at the center of their racks. A capillary 15 cm long is then inserted in the pipet holder, and the holder is placed in the clamp of the manipulator so that it is held close to the capillary. The correct position of the manipulator is now found by moving it until the free end of the capillary is approximately 10 mm to the left (referring to Fig. 45) of the optic axis of the microscope and about 1 cm above the stage. The height of microscope or manipulator may be adjusted by means of additional boards. When the correct positions have been found, their rigid maintenance is insured by means of tightly fitting blocks. If a cover is available which can be made to fit the baseboard, the apparatus may be kept assembled and ready for use over any length of time.

It is obvious that the base should be mounted on a low table which permits the operator to be comfortably seated while working and observing. If the light for the illumination of the microscopic field is sufficiently strong it is possible to project an image 5 to 10 cm in diameter on a piece of white bristolboard which may be mounted inside a black box and a short distance above the eyepiece as indicated in Fig. 45. The observation of the screen image produces less eye-strain than viewing through the eyepiece. Another advantage is that the height of the table may then be adjusted so as to give a maximum of comfort during the operation of the various controls. The screen is mounted on the tube of the microscope in such a manner that it always remains at a fixed distance above the eyepiece and may be simply swung aside whenever direct observation becomes necessary. The eyepiece micrometer is focused on the screen by adjusting the eye lens after the object has been focused. The focusing may be continued, if necessary, by alternately adjusting the position of the microscope tube and of the eye lens until scale and object simultaneously appear in sharp outlines. The value of the scale divisions will be approximately the same as under direct observation. It is preferable, however, to calibrate the eyepiece micrometer under the conditions of use.

**Microscope Lamps.** Two microscope lamps are required. One is placed in front of the microscope to serve for observation with transmitted light. The light is sent in the direction of the arrow *T*, Fig. 45, to the mirror of the microscope. The second lamp is used for observation with reflected light, arrow *R*. The lamp is placed to the left of the microscope so that it is on a level with the stage. The light is sent

horizontally into the moist chamber on the stage, and the lamp must permit collecting the light into a narrow pencil not more than 6 mm in diameter at the focus. Switches for both lamps in a handy location permit rapid interchange of observation with transmitted and reflected light.

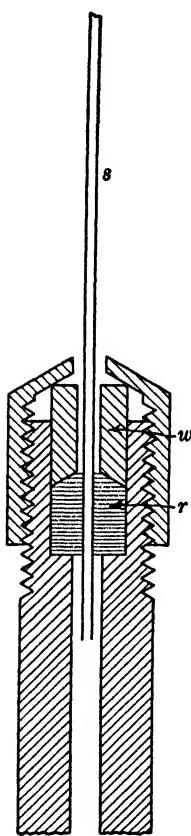


FIG. 46.

Pipet holder, approximately three times natural size; *s*, shank of micropipet; *r*, rubber tubing; *w*, metal washer.

**Pipet Holder.** The pipet holder is a metal tube of 1- or 2-mm bore, about 4 mm in outer diameter and approximately 12 cm long, which is fitted with rubber washers *r*, metal washers *w*, and screw caps at both ends, Fig. 46. The rubber washers consist of short pieces of thin, flexible rubber tubing. When the screw cap is tightened, the rubber tubing is compressed between the concave face of the advancing metal washer and the bottom of the well provided for the washers. This produces a tight seal around the shank *s* of the inserted micropipet. The fine copper tubing *t*, Fig. 45, is attached similarly to the other end of the pipet holder. Pipet holder and attached copper tube are best obtained from one of the optical firms which manufacture micromanipulators. The pipet holders supplied by E. Leitz for the micromanipulator of R. Chambers are very satisfactory.

**Plunger Device.** The plunger device I, Fig. 45, may be made of brass or stainless steel. The copper tubing is connected to a cylinder of 14-cm length and 5-mm bore. The plunger is 4 mm in diameter, and it is threaded with about 40 threads to the inch for half its length. The vertical wing close to the milled head has the female screw which controls the motion of the plunger. The plunger is made tight in the cylinder by means of a leather packing and a large packing nut as employed by Johnson and Shrewsbury<sup>48</sup> with their mercury control for capillary burets. Plunger device, copper tubing, and pipet holder are completely filled with water so that no air bubbles remain behind.

This operation is much facilitated if the cylinder of the pressure device is provided with a side tube (and stopcock) close to the leather packing of the plunger. The water is boiled to eliminate dissolved gases and introduced while still lukewarm. The plunger is nearly

<sup>48</sup> D. L. Johnson and Ch. L. Shrewsbury, *Mikrochemie*, **26**, 143 (1939).

completely withdrawn from the cylinder so as to admit a maximum volume of the water. The pressure device is secured to the baseboard, and the copper tubing, which should not be shorter than 1 meter, is bent to form two or three helical turns to obtain the required freedom of motion for the pipet holder.

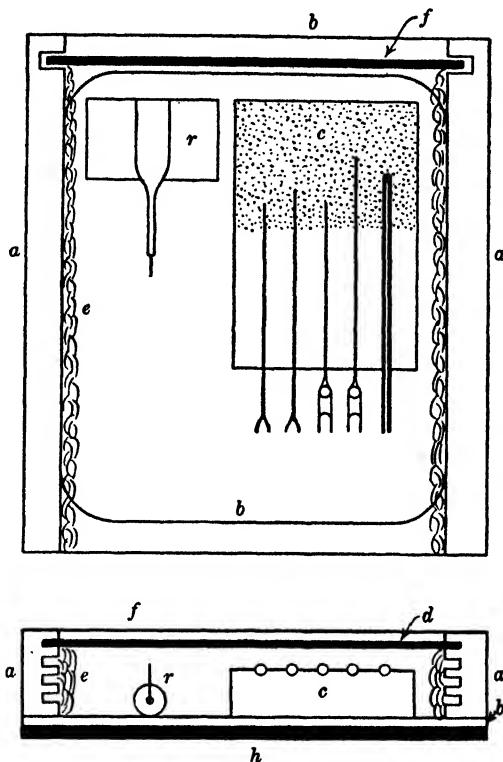


FIG. 47. Mois chamber, natural size, top and front views; *c*, carrier with capillary cones, reagent containers, and measuring capillary; *r*, condenser rod; *e*, cotton lining.

**Two Chambers.** Figure 47 shows top and front views of the chamber designed by Cefola.<sup>46</sup> The bottom is formed by a glass plate *h*, 60 mm by 68 mm. The brass frame *b*, which forms a border approximately 5 mm wide all around, is attached to the plate *h* with DeKhotinsky cement. The brass rods *a*, which form the sides of the chamber, are held to the frame *b* by means of screws. The bars are 6.7 cm long, 6 mm thick, and 11 mm high. The top of the cell is formed by a thin glass plate, *d*, approximately 52 mm by 63 mm, which fits the horizontal grooves of the bars *a* so that it slides freely

but not too loosely. A narrow strip of thin glass plate *f*, 11 mm by 53 mm, fits vertical grooves of the bars *a* and forms the back of the cell. To obtain a moist chamber, the horizontal grooves of one cell are lined with cotton *e* which is kept moistened with water. The other cell which is to be used as "dry chamber" does not receive the cotton lining.

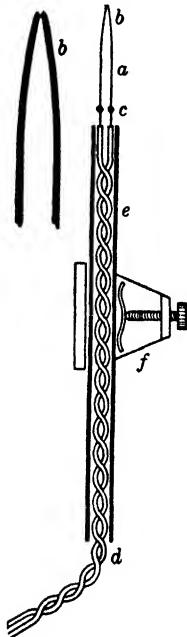


FIG. 48. Heating element.

the clamp *f* of the manipulator. To prevent the wire from twisting around in the tube, insulating tape is applied at *d*. The ends of the insulated copper wire are connected to a variable transformer which is connected to the alternating-current line. In general, not more than 5 volts need be supplied to the heating element.

**Gloves.** A pair of gloves or four finger cots for thumbs and index fingers are necessary. Rubber gloves are quite inconvenient; thin cotton or silk ones are preferable. They are kept ready in an envelope and must be meticulously clean.

**Forceps.** Two forceps, preferably of stainless steel and having polished flat tips, are required. The tips of one of them should be lined with cork (Appendix).

**Reagent Containers.** A large number of reagent containers is prepared from capillary tubing of 0.5- to 1-mm *uniform* bore. It is un-

derstood that the capillary tubing must be clean. The outside is wiped first with a piece of moist flannel and then with chamois. Without being touched with the fingers, the tube is then placed on a clean paper. Gloves are worn during the following operation. The capillary is fused to a bead 2 cm from one end, and the bead is drawn out to a rod approximately 0.3 mm in diameter and 2 to 5 cm long. Cutting at the proper points gives two reagent containers, Fig. 49A, B, C, and

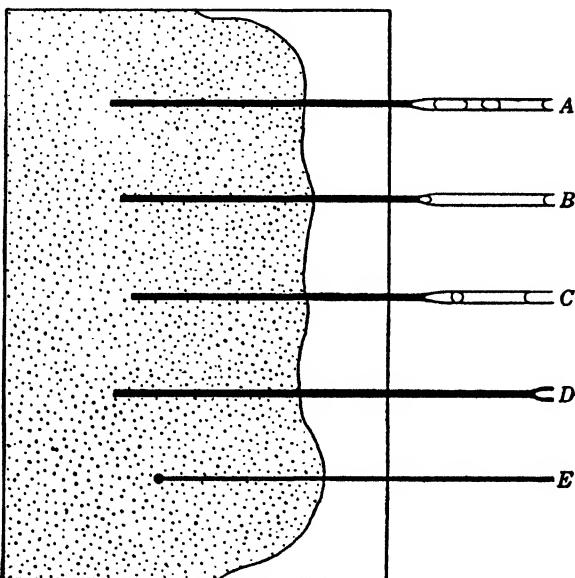


FIG. 49. Carrier with reagent containers A, B, C, capillary cone D, and measuring capillary E. Twice natural size.

each consisting of a 2- to 3-cm length of the rod with 5 to 8 mm of the original capillary attached. The work is continued until ten or twenty reagent containers have been made. Then the bore of at least one container out of each pair obtained is measured as described in Expt. 8. The maximum deviation from the arithmetical mean of all values should not be more than 50 parts per 1000. The reagent containers are placed in a screw-cap vial. The average diameter of the bore is used to calculate the length of the containers which holds 1 ml of liquid. This length is expressed in microns and in values of the eyepiece micrometer used with the microscope. These data are recorded on a label which is attached to the vial.

**Capillary Cones.<sup>49</sup>** The cone, Fig. 50, has a capacity of approximately  $0.5 \lambda$  and is made from a thin-walled capillary of about

<sup>49</sup> A. A. Benedetti-Pichler, *Ind. Eng. Chem., Anal. Ed.*, 10, 483 (1937).

0.5-mm bore. Gloves are worn, and capillaries are freshly drawn out from *clean* glass tubing and cut into pieces 6 to 10 cm long. After the bore has been checked cones are prepared by heating the center portion of a piece of capillary in the edge of a non-luminous Bunsen flame until the glass fuses together, forming an elongated bead. Outside the flame, the bead is drawn out to a rod approximately 0.3 mm in diameter and 2 to 5 cm long. Cutting in the proper places gives two capillary cones with handles. The length of the cone proper should be 2 mm, and half this length should be formed by the quite *blunt* taper of the cone. The handle is given a length of 2 cm. The finished capillary cones are collected in a screw-cap vial. They must never be touched with the fingers, because when the apparatus is used

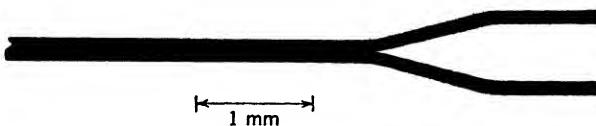


FIG. 50. Capillary cone.

in the moist chamber fingerprints develop into a pattern of small droplets which greatly interferes with the microscopic observation of the contents.

**Measuring capillaries**, Fig. 49E, permit a more accurate determination of very small volumes of solutions (1 to 10  $\text{m}\lambda$ ) than reagent containers. They are made by drawing out thin-walled capillaries of 0.8- to 1-mm bore to fine capillaries of 0.05- to 0.2-mm bore. The fine capillaries are cut into lengths of 2 to 3 cm each. Gloves are worn so that the capillaries will not be touched with the fingers. The diameter of the bore is measured at both ends of the short pieces of capillary and recorded. One end of each piece of fine capillary is fused shut. The measuring capillaries thus obtained may be kept in pieces of wide capillary, sealed at both ends.

**Condenser Rod.**<sup>46</sup> Side and top views of this device are shown in Fig. 51. It consists essentially of a short piece of glass rod which tapers rapidly to a fine thread. The thread is cut to provide a tiny platform *d* for the performance of confirmatory tests. A strong beam of light is sent into the rod through the base *f*. Most of the light is collected in the thread *c* and emerges at *d*, providing efficient illumination for the observation of the test.

A soft-glass rod 4 to 5 mm in diameter is selected. The glass should have as little color as possible. The rod is drawn out to a thinner rod of approximately 2-mm diameter so that a rather quick

taper between *a* and *b*, Fig. 51, is obtained. At a distance of 10 mm from the taper, rod *b* is drawn out to a thread approximately 0.3 mm in diameter. The thread is broken 2 cm from the taper. The thick part of the rod is cut so as to give *a* a length of 10 to 12 mm. After scratching with a file, the rod is broken while part *a* is held with pliers. It is essential that an even surface is obtained at *f*. The thread *c* is now bent at a right angle by approaching with it the edge of a small

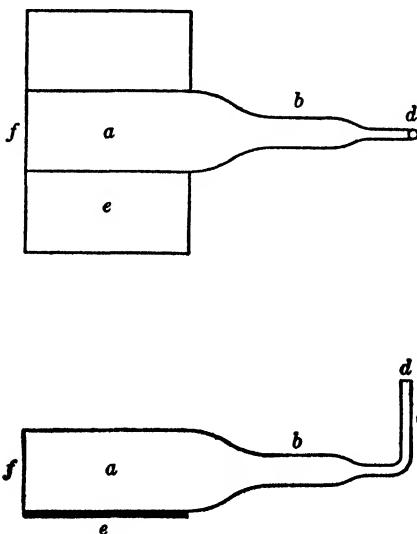


FIG. 51. Condenser rod, twice natural size.

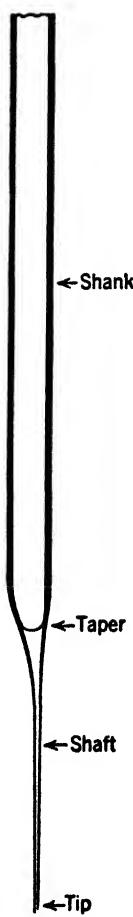
Bunsen flame while the rod is held horizontally. The thread bends by its own weight until it is at right angles to *a* and *b*. The rod being held at *a*, thread *c* is dipped into molten paraffin nearly up to the bend and then slowly withdrawn. When the paraffin on the thread has solidified, the thread *c* is scratched 5 mm above the bend and broken off with the help of forceps. The break should be clean and, if possible, at a right angle to the axis of the thread. Rod *a* is finally attached to a cover slip *e* by means of some Duco household cement so that thread *c* assumes a perpendicular position when *e* is supported horizontally.

The platform *d* should have a diameter of approximately 0.3 mm, which corresponds to an area of approximately  $0.07 \text{ mm}^2$ . The film of paraffin on the cylindrical surface of the thread prevents solutions placed on the platform *d* from flowing down the sides of the thread, and the solutions are thus confined to the small area of the platform.

**Micropipets.** The micropipet proper consists of the shaft and the tip, Fig. 52. The small volumes of solutions employed will hardly ever fill more than the shaft and part of the taper. The shank contains

the air cushion which serves to separate the solution in the micropipet from the water used to transfer the pressure from the plunger device. The micropipets are made from capillaries 0.5 to 1 mm in outside diameter, which are drawn from soft-glass tubing of 6-mm bore and 8-mm outer diameter. The outside diameter of the capillary should be somewhat less than the bore of the metal washer of the pipet holder. The micropipets may be drawn out by hand with the aid of the microburner shown in Fig. 1. The flame should have the size of a pinhead. A piece of capillary approximately 15 cm long is held horizontally in front of the experimenter. Its right-hand end is grasped with a pair of flat forceps, and thumb and index finger of the left hand seize it at a point 5 cm from the forceps. The hands are steadied by resting the outer, fleshy parts of the palms on the table top, and the capillary is brought over the microflame so that it is heated approximately 1 cm from the forceps. A slight pull is applied immediately so that the drawing of the pipet starts when the glass begins to soften. A steady horizontal pull is maintained with both hands so that the cool parts of the capillary move symmetrically to the right and left while the hot part always remains above the microflame. The operation takes less than one second, and it is finished when the capillary snaps at the center of the drawn-out portion.<sup>50</sup> Inspection will indicate variations of the technique required to obtain a micropipet of the proper shape. The shaft should have a length of 5 to 7 mm, and it should taper gradually as shown in Fig. 52. The opening at the tip should have a diameter from 30 to 40  $\mu$ . Often the tip will be too fine, and frequently it is fused shut when the pipet is drawn. A short length of the tip is then snipped off with a pair of forceps so as to give the required opening. The shank of the micropipet is cut to a length of 10 to 12 cm. A supply of five to ten micropipets is prepared and stored in a stoppered test tube.

FIG. 52.  
Micropipet,  
approximately  
six times  
natural size.



<sup>50</sup> Ruth B. Howland and M. Belkin, *Manual of Microscopy*, New York, New York University Press Book Store, 1931.

The preparation of micropipets is greatly facilitated by one of the mechanical devices described by Du Bois<sup>51</sup> and Rachele.<sup>26</sup>

**Reservoir of Water for Cleaning of Micropipets.** The reservoir *C*, Fig. 45, is given the shape of a short pinchcock buret. A glass bead serves for the control of the outflow. The inside of the rubber tubing is cleaned with a brush and soap solution before being attached to the tube. The reservoir is filled with distilled water and then covered with an inverted vial to keep the dust out.

## EXPERIMENT 56

### Silver Dichromate on the Platform of the Condenser Rod<sup>46</sup>

L. I., 1 my Ag or less

Approximately 1 mλ of acidulated silver solution is transferred to the platform of the condenser rod and there treated with an equal volume of saturated potassium dichromate. The experiment is performed in the moist chamber to prevent evaporation of the test drop. It is assumed that the apparatus has been assembled as shown in Fig. 45 and that the plunger device, copper tubing, and pipet holder have been filled with water so as to eliminate air bubbles. It is further assumed that the micrometer scale of the eyepiece has been calibrated and that a supply of reagent containers and measuring capillaries is available.

**INSERTING MICROPIPET INTO PIPET HOLDER.** The plunger of the pressure device is advanced until water appears at the opening of the pipet holder. The shank of the micropipet is inserted in the opening, and the screw cap is made tight. If necessary, the plunger is advanced until the meniscus of the hydraulic water in the shank of the micropipet is visible at approximately 3 cm from the opening of the pipet holder. The micropipet is now ready for use.

**ASSEMBLING APPARATUS IN MOIST CHAMBER.** Two reagent containers and one measuring capillary are selected, and the number of scale divisions of the eyepiece micrometer which correspond to a volume of 1 mλ in the reagent containers and in the measuring capillary, respectively, are calculated. By means of capillary pipets one reagent container is filled with silver test solution (10 mg Ag per ml) which has been acidified so that it contains 10 per cent nitric acid, and the other with a saturated solution of potassium dichromate. The tubes are assembled side by side on the carrier as shown in Fig. 47. The handles of the reagent containers and the sealed end of the measuring capillary are pushed into the layer of vaseline. It is advisable to place the tubes parallel to the long edge of the carrier and not more than 1 mm apart. Later operations are facilitated if the openings of all

<sup>51</sup> Delafield Du Bois, *Science*, 73, 344 (1931).

tubes lie in a straight line parallel to the long edge of the carrier. Special attention must be paid to an orderly arrangement. The measuring capillary should be placed close to the condenser rod. If many reagents are needed, it is helpful first to prepare a sketch in which the containers are labeled, and then to proceed according to this plan.

The cover slide of the moist chamber is removed and, if necessary, water is added to the cotton lining. Two small drops of water are placed on the bottom plate of the chamber at the locations indicated by the letters *r* and *c* in Fig. 47. Then the carrier and a condenser rod with a platform of approximately 0.3-mm diameter are brought into the positions shown in the figure. The water droplets spread between the glass surfaces, and the surface tension holds carrier and condenser rod in their assigned places. The moist chamber is closed by sliding the cover plate into the uppermost groove, and it is then clamped into the mechanical stage. The stage of the microscope is rotated so that the opening of the moist chamber faces the manipulator on the right-hand side of the microscope and the controls of the mechanical stage are on the left-hand side of the microscope, Fig. 45.

**INTRODUCING THE MICROPET INTO THE CHAMBER.** Using transmitted light and a magnification of the microscope of 40 to 60, the opening of the reagent container with the silver solution is focused so that a sharp image of the plane containing the axis of the capillary is produced. This particular type of focus is easily recognized by watching the outer contours of the walls of the capillary. They must appear as perfectly sharp straight lines. The setting of the microscope is retained during the following operations.

The reagent container is withdrawn by means of the mechanical stage so that only one-third of the field of vision is occupied by the container, Fig. 53. The manipulator is now swung around until the micropipet is parallel to the axes of the reagent containers. Observing with the unaided eye and using the controls of the manipulator, one introduces the micropipet into the moist chamber and brings its tip close to the opening of the reagent container. Through the microscope (or on the screen) a blurred image of the tip of the micropipet will be perceived. Without touching the adjustment of the microscope, the tip is brought into focus by raising or lowering the micropipet with the manipulator. This operation also brings the tip of the micropipet into the horizontal plane which contains the axis of the reagent container. The tip of the pipet and the axis of the container are then made parallel by rotating the stage of the microscope. By operating the side motions of the manipulator the tip of the pipet is finally brought into the axis of the reagent container, and the opening of the

pipet is advanced just to the opening of the reagent container. The positions of pipet and container should now appear in the image as shown in Fig. 53.

**WITHDRAWING MEASURED VOLUMES FROM THE REAGENT CONTAINER.** Assuming that calculation has shown a column of liquid of a length of 0.5 division of the micrometer scale to have a volume of 1 ml, one will withdraw from the container a column of approximately 1 division length so as to obtain somewhat more than the required volume.

The micrometer eyepiece is rotated to make the scale appear parallel to and in the image of the reagent container. Then the left hand grasps the control of the mechanical stage, and the right hand the control of the plunger device. The reagent container is moved forward by means of the mechanical stage until the opening of the micropipet is immersed in the silver solution beyond that length which is to be taken up by the pipet. As a rule, solution enters the micropipet as soon as it touches the liquid, and the solution must be completely expelled from the pipet before the position of the reagent container is finally adjusted. If the plunger device operates properly and hydraulic water fills pressure chamber, copper tubing, and pipet holder without air bubbles, the meniscus in the micropipet will follow every move of the plunger. Thus, the meniscus is forced close to the opening of the pipet, and the position of the reagent container is adjusted until the meniscus in the reagent container coincides with a convenient division of the micrometer scale, Fig. 54. Then suction is applied carefully by withdrawing the plunger from the pressure chamber so that the meniscus recedes slowly in the reagent container. When the meniscus in the reagent container has traveled through the desired number of scale divisions (1 division corresponding to the assumption made at the outset, i.e., from "10" to "11" in Fig. 54), the reagent container is rapidly withdrawn by means of the mechanical stage. The pipet now contains approximately 2 ml of the silver test solution.

**USE OF THE MEASURING CAPILLARY.** The micropipet is backed up horizontally for approximately 1 cm by means of the manipulator.

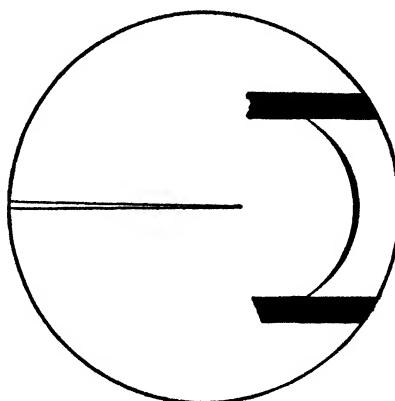


FIG. 53. Microscopic image of pipet ready to enter reagent container.

Then with the use of the mechanical stage, the measuring capillary is brought into the field of vision so that it extends to the center of the field. The microscope is focused on the axial plane of the measuring capillary, and then the opening of the capillary is approached with the tip of the micropipet while observing with the unaided eye. The position of the pipet is corrected until a blurred image of the tip appears in the microscopic field. The tip of the micropipet is then brought into focus by means of the vertical adjustment of the manipulator. The stage of the microscope is rotated to make pipet and meas-

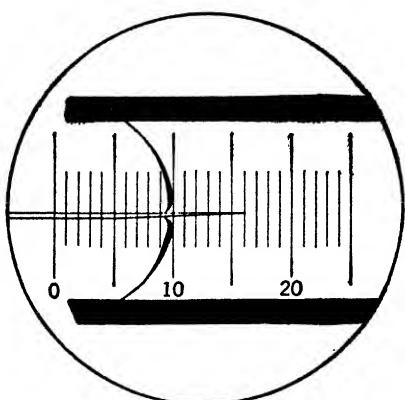


FIG. 54. Measuring solutions in the reagent container.

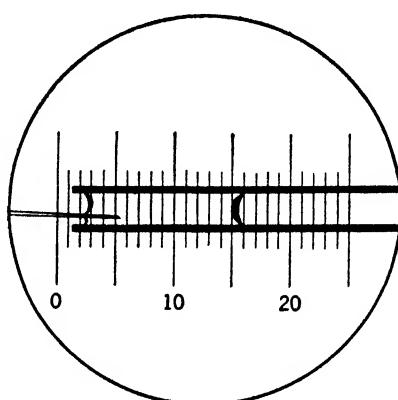


FIG. 55. Use of measuring capillary.

uring capillary parallel, and the horizontal motions of the manipulator are utilized to bring the tip of the micropipet into the axis of the measuring capillary and to approach the opening of the capillary with the point of the pipet. A slight rotation of the eyepiece will make micrometer scale and capillary perfectly parallel.

The measuring capillary is now advanced by means of the mechanical stage so that the opening of the pipet enters the measuring capillary to at least three-fourths of the length of the liquid column to be measured off. Assuming that 12 divisions of the micrometer scale correspond to 1  $\text{m}\lambda$ , the opening of the pipet should be located at division 11 or 12 of Fig. 55.

In general, it does not matter whether or not the tip of the micropipet touches the wall of the measuring capillary. When the plunger is advanced into the hydraulic chamber, the silver solution first forms a droplet at the opening of the pipet. The droplet grows and finally fills the bore of the capillary so that two menisci appear in the measuring capillary. Again, the left hand operates the controls of the

mechanical stage, and the right hand turns the milled head of the plunger device. The plunger is *slowly* advanced into the hydraulic chamber so that the two menisci in the measuring capillary move slowly apart as the volume of solution in the capillary increases. Simultaneously, the measuring capillary is slowly backed up by means of the mechanical stage so that only a short length of the tip of the pipet is immersed. Under slight pressure and suction supplied by the plunger device, the length of the liquid column in the measuring capillary is finally adjusted so that the two menisci are the proper distance (assumed 12 divisions) apart, Fig. 55. Then the measuring capillary is quickly withdrawn so that its opening remains just visible near the edge of the field. The measuring capillary now contains 1 ml of the silver test solution.

**EMPTYING THE MICROPIPET.** The micropipet is completely withdrawn from the moist chamber by means of the manipulator. A strip of filter paper is grasped with the fingers by one end. The other end is touched to the opening of the pipet, and the plunger is slightly advanced so that all the silver solution is expelled from the micropipet. It may be worth while at this time to check on the position of the meniscus of the hydraulic water in the shank of the pipet, and to correct it, if necessary. This meniscus should always remain within the third of the shank close to the pipet holder.

**REMOVING SOLUTION FROM THE MEASURING CAPILLARY.** The microscope is still focused on the axis of the measuring capillary. The micropipet is brought into the field of vision and into focus by means of the vertical motion of the manipulator. It is then aligned with the axis of the measuring capillary and advanced into the field of vision so that the opening of the pipet is located approximately 10 scale divisions in front of the mouth of the measuring capillary. The mechanical stage being operated with the left hand, the measuring capillary is advanced toward the pipet so that the opening of the pipet is continuously only a few divisions inside the near meniscus of the silver solution in the capillary. Suction is applied very gradually with the plunger device. When nearly all the liquid has been taken up, the motion of the plunger may be reversed so as to stop the intake into the pipet, and the small remainder of liquid in the measuring capillary is then taken slowly into the pipet by a very slight withdrawal of the plunger. The measuring capillary is finally withdrawn by means of the mechanical stage.

**CALIBRATION OF MICROPIPET.** Calibration of micropipets may be attempted for small volumes of solutions. Low magnifications giving diameters of the field of vision from 0.5 to 2 mm are desirable.

The micropipet is advanced into the field of vision by means of the manipulator so that the opening of the tip and the other meniscus of the silver solution are located within the eyepiece micrometer scale. One meniscus must be located at the opening of the pipet, and it may be brought into this position by means of the plunger device. The distance from the opening of the pipet to the other meniscus corresponds to a volume of approximately 1 ml and is recorded for future use.

**TRANSFER OF THE TEST SOLUTION TO THE PLATFORM OF THE CONDENSER ROD.** The micropipet is withdrawn to a distance of 1 cm from the axis of the microscope. Then, observing with the unaided eye, the platform of the condenser rod is brought into the field of vision and focused. The lamp sending the light to the mirror of the microscope is turned off, and a strong beam of light is sent from the lateral lamp to the base of the condenser rod. The lamp is adjusted until the platform is brightly illuminated while the rest of the field of vision is dark. This type of illumination facilitates the observation of the colors and precipitates obtained in the confirmatory tests, and the position of the lateral lamp is retained for future use. For the following manipulations it is preferable to add general illumination with transmitted light.

By means of the manipulator and while observing with the unaided eye, the tip of the micropipet is brought close to the platform of the condenser rod, which is in the focus of the microscope. Then the microscopic image is watched and the opening of the pipet is brought into sharp focus and close to the platform by means of the manipulator, Fig. 56a. Always operating the controls of the manipulator only, the tip of the pipet is slightly raised so that the outlines lose somewhat in sharpness and then moved horizontally so that the opening of the pipet appears above the side of the platform, Fig. 56b. The pipet is then lowered so that the opening touches the surface of the platform when the outlines of the tip become sharp, Fig. 56c. The plunger is very slowly advanced into the hydraulic chamber so as to expel the contents of the micropipet slowly. When the first bubble of air appears at the opening of the pipet, the pipet is first raised and then completely withdrawn from the moist chamber.

**CLEANING THE MICROPIPET.** The valve of the water reservoir is operated so that a large drop of water forms at the tip of the outlet. The tip of the micropipet is inserted in this drop by means of the manipulator, and then suction is applied with the plunger device. When the water has filled the shaft and part of the taper of the micro-pipet, the pipet is withdrawn from the hanging drop and its contents

are expelled by touching filter paper to the opening of the micropipet and reversing the motion of the plunger. The cleaning operation is repeated two or three times, each time with a fresh drop of water. Finally the position of the meniscus of the hydraulic water in the shank of the pipet is corrected, if necessary.

**MEASURING THE REAGENT.** The saturated potassium dichromate solution may be measured off in the reagent container. As closely as possible 1 ml of it may be taken up into the micropipet by the procedure described for the approximate measurement of the silver test solution. This procedure is quite accurate for large volumes of 10 ml or more. The measurement of a small volume of solution, as 1 ml or less, is more accurately based on the above-described calibration of the micropipet. The tip of the pipet is inserted in the dichromate solution so that the opening of the pipet coincides with the far end of the micrometer scale. The solution is allowed to enter the pipet until that length of the shaft is filled which corresponds to a volume of 1 ml.

**TRANSFER OF THE REAGENT TO THE PLATFORM AND OBSERVATION OF THE TEST.** The transfer to the platform is performed as described for the silver test solution. It is advisable, however, to supply slight pressure by means of the plunger device and to cut off the general illumination with transmitted light just before lowering the tip of the micropipet into the test solution on the platform. The pressure on the solution in the micropipet causes it to flow out of the pipet as soon as the tip makes contact with the silver solution on the platform, and separation of a precipitate inside the tip is prevented. Absence of general illumination of the field improves the observation.

The micropipet is quickly removed when a bubble of air appears at the opening of the pipet, indicating that the reagent is completely expelled. The precipitate is observed in the illumination furnished by the condenser rod. As a rule, some crystals are found which exhibit the shape and color characteristic for silver dichromate.

The micropipet is cleaned immediately after withdrawal from the moist chamber. The platform may be prepared for further use by rinsing with 10 per cent nitric acid and distilled water. If this is not satisfactory, a new platform is easily obtained by cutting the thread close below the old platform. This can be repeated several times be-

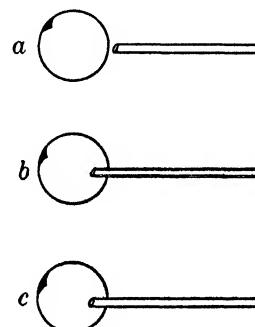


FIG. 56. Depositing solutions on the platform of the condenser rod, approximately thirty times natural size.

fore it becomes necessary to reject the condenser rod. The reagent containers and the measuring capillary are rejected after removal from the carrier.

### EXPERIMENT 57

#### Separation of Antimony and Bismuth in the Capillary Cone<sup>48, 49</sup>

Close to 10 ml of acid solution containing 0.1 γ of antimony and 0.01 γ of bismuth is treated with hydrogen sulfide. The volume of the precipitate is estimated, and the precipitate is then treated with sodium sulfide reagent. The volume of the residue of bismuth sulfide is estimated, and the solution containing the antimony is transferred to another capillary cone where it is treated with acid. The volume of the precipitate of antimony sulfide is estimated. The sulfide precipitates are dissolved in acid, and small portions of the solutions are used for confirmatory tests.

**ASSEMBLING APPARATUS IN THE MOIST CHAMBER.** Across the long edge of a carrier (Fig. 49) are assembled in the order listed: five reagent containers, one measuring capillary, and two capillary cones. The reagent containers are filled with: (a) known solution V, being 5 M in hydrochloric acid and containing 10 mg Sb per ml and 1 mg Bi per ml; (b) distilled water; (c) 0.12 M nitric acid; (d) sodium sulfide-hydroxide reagent; (e) 0.5 M sulfuric acid. The carrier is placed in the moist chamber, which is then closed.

**MEASURING THE TEST SOLUTION.** Approximately 15 ml of the known solution is taken from the reagent container into the micropipet, and exactly 10 ml of it is transferred to the measuring capillary. The micropipet is emptied on a strip of filter paper, after which the solution in the measuring capillary is taken up into the micropipet. This procedure should always be followed when small volumes of a solution under investigation are to be measured. As a substitute, the solution may be measured in the shaft of the micropipet as outlined in Expt. 56.

**TRANSFER OF SOLUTION TO THE CAPILLARY CONE.** The first capillary cone is brought into the field of vision, and the microscope is focused on the point of the taper, which, of course, lies in the axis of the cone. The capillary cone is somewhat backed up so that its opening is situated close to the center of the field. The tip of the micropipet is brought close to the opening of the cone, focused by means of the manipulator, and aligned with the axis of the cone. The opening of the pipet is advanced with the manipulator to a point just inside the opening of the capillary cone, and then the cone is advanced by means of the mechanical stage until the opening of the micropipet touches the wall of the cone close to the point of the taper,

Fig. 57. Slight pressure is now supplied with the plunger device so that the contents of the pipet are slowly delivered to the point of the cone. The pressure must be supplied carefully and slowly, for otherwise air bubbles, which follow the solution, will cause splashing and a considerable portion of the solution will be spattered on the sides of the cone, away from the tapered end, where it may not come in contact with the reagents to be added later. If an accident of this kind should happen, it is best to collect all the liquid in the point of the

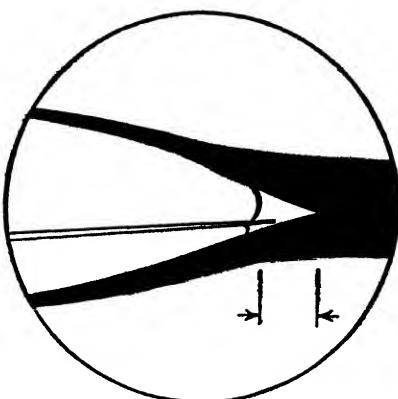


FIG. 57. Transferring solutions to the capillary cone, approximately fifty times natural size.

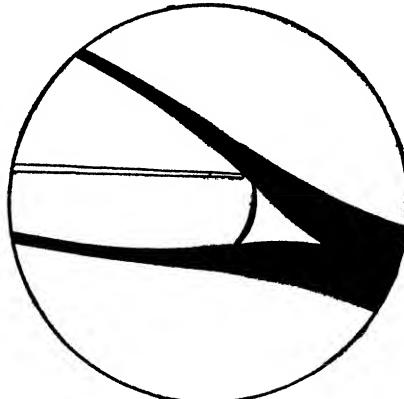


FIG. 58. Delivering solution to the wall of the capillary cone.

capillary cone by means of the centrifuge. The safe procedure to follow, however, is to stop supplying pressure when the meniscus in the pipet comes close to the opening. The capillary cone is then backed up until the tip of the pipet is just outside the liquid in the cone. By rotating the stage, the capillary cone is tilted at an angle of approximately 30 degrees to the pipet, and by operating the mechanical stage, the opening of the pipet is made to touch the wall of the cone close to the solution as shown in Fig. 58. By exerting slight pressure with the plunger the remainder of the solution is expelled from the tip of the micropipet and the capillary cone is immediately withdrawn by means of the mechanical stage.

The micropipet is removed from the moist chamber and cleaned by two rinsings with 6 *M* hydrochloric acid followed by two rinsings with distilled water. The acid is supplied as a drop hanging from the end of a glass rod.

**DETERMINATION OF VOLUME IN THE CAPILLARY CONE.** The capillary cone now contains 10 ml of solution. The distance from the

point of the taper to the meniscus of the solution is measured with the eyepiece micrometer and recorded for future use. The distance in question is indicated in Fig. 57.

**DILUTING WITH WATER PREVIOUS TO TREATMENT WITH HYDROGEN SULFIDE.** Approximately 90 ml of distilled water is taken from the second reagent container into the micropipet and immediately transferred to the test solution in the first capillary cone.

**ADDING REAGENTS TO SOLUTIONS IN THE CAPILLARY CONE.** The pipet is introduced into the capillary cone as outlined above. Before the tip of the pipet is actually immersed in the test solution, slight pressure is applied with the plunger device so that the outflow of water starts as soon as the tip of the pipet makes contact with the solution in the cone. Otherwise some of the test solution might enter the pipet, and separation of a precipitate might clog the tip of the pipet. All but a small amount of the water which is retained in the tip of the pipet is expelled. Formation of a dark precipitate is observed through the microscope. If, however, the light from the mirror of the microscope is cut off and the light of the lamp on the side of the stage is concentrated on the capillary cone, the white color of the oxychlorides is revealed.

**STIRRING THE CONTENTS OF THE CAPILLARY CONE.** The contents of the cone could be efficiently stirred by inserting the pipet deeply into the solution and expelling a stream of air bubbles from the opening of the tip. This method is not applicable, because of the resulting spattering of the contents over the walls of the cone. The solution might be collected again in the point of the cone by means of the centrifuge, but much of the *precipitate* would remain on the walls. A satisfactory stirring effect may be obtained by moving the shaft of the pipet, which is sufficiently sealed by the presence of the last trace of the reagent in the tip, through the contents of the cone by means of either the mechanical stage or the manipulator. Very efficient stirring is obtained by plucking with the forefinger the copper tubing connecting pipet holder and plunger device. This imparts a vibrating motion to the tip of the micropipet which is immersed in the contents of the capillary cone. The last of the water remaining in the tip of the pipet is finally expelled on the wall of the cone close to the meniscus of the liquid in the cone as described in connection with the transfer of the test solution.

The micropipet is completely withdrawn from the moist chamber and may again be cleaned with hydrochloric acid and water. Cleaning is not essential at this time, however.

**TREATING THE CONTENTS OF THE CAPILLARY CONE WITH HYDROGEN SULFIDE.** Simple calculation shows that somewhat less than  $0.3 \lambda$  of hydrogen sulfide, measured under normal conditions, is required for the precipitation of  $1 \gamma$  of antimony as sulfide. The following procedure obviously insures the presence of a sufficient excess of the gas to give complete precipitation.

A glass tubing is drawn out to a capillary of approximately 1-mm bore, which is then bent as shown in Fig. 59. A plug of cotton is placed into the tube *b*. The capillary is squarely cut open at *a* so as to permit introducing the capillary cone, and it is slightly drawn out at *c*.

A piece of hot cloth or lens paper is held ready on a heating block or in a drying oven. The moist chamber is opened for a moment, and the first capillary cone is lifted out by means of cork-tipped forceps. The vaseline adhering to the handle of the capillary cone is quickly removed with the warm

cloth, and the capillary cone is immediately inserted in the capillary shown in Fig. 59, which is then drawn out to a fine tip at *a*. The wide tube *b* is connected to a hydrogen sulfide generator, and the air in the capillary is displaced by hydrogen sulfide. When the odor of the escaping hydrogen sulfide becomes distinctly noticeable at *a*, the point of the tip is sealed. The capillary is then fused shut at the constriction *c*, drawn off from the wide part of the tube, and placed for half a minute in water at approximately  $70^\circ \text{ C}$ , which is held ready in a beaker. The separation of an orange precipitate can be observed with the unaided eye. The capillary is removed from the water bath, and the precipitation is allowed to stand for 5 minutes at room temperature. Then the capillary is cautiously cut open at *d*. By slight stroking with a file the handle of the capillary cone

Fig. 60. Working in the capillary cone: *A*, centrifuging; *B*, heating.

is made to protrude from the opening of the wide capillary so that it can be grasped.

**COLLECTING PRECIPITATES BY MEANS OF THE CENTRIFUGE.** A capillary of 0.6- to 0.7-mm bore is cut to a length of 6 to 7 cm. The handle of the capillary cone protruding from the hydrogen sulfide cap-

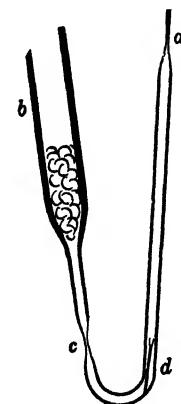
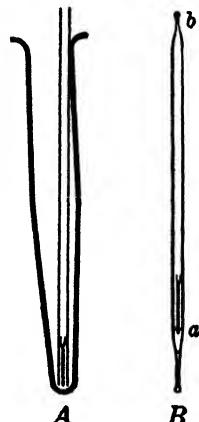


Fig. 59. Treatment with hydrogen sulfide.

illary is grasped with the fingers. The cone is withdrawn and immediately introduced, opening first, into one end of the capillary just prepared. The latter capillary is held horizontal and placed in a microcone which is then transferred to the shell of the centrifuge, Fig. 60A.

An electric centrifuge is advisable, but not indispensable. Because of the following estimation of the volume of the precipitate, it is essential, however, that the centrifuge is always made to run at the same speed for the same length of time. Half a minute at 2000 or 3000 revolutions will be satisfactory. If the centrifuge is driven by hand it is advisable to count the turns of the crank; experience will show how many turns are required. Any deviation from the adopted standard procedure should be recorded for future reference.

Holding the microcone horizontally, one withdraws the capillary. The handle of the capillary cone usually protrudes from the opening of the capillary. It is grasped with the fingers, and the capillary cone is pulled out of the capillary. The capillary cone is now taken with cork-tipped forceps and returned to its former position on the carrier. The moist chamber is immediately closed again.

The wide capillary is reserved for further use. Its purpose is two-fold. Aside from facilitating the handling of the capillary cone, it limits the air space around the capillary cone sufficiently to prevent excessive evaporation of the solutions contained in the cone. The same principle is applied when solutions in the capillary cone are either heated or to be treated with gases, Figs. 59, 60A and B.

**ESTIMATION OF THE VOLUME OF PRECIPITATE.** The color of the precipitate is observed with lateral illumination. Observation with transmitted light is used for the estimation of the volume of precipitate, and the microscope is focused on the point of the taper of the capillary cone. The precipitate is usually found to occupy an area *A* similar to that indicated by shading in Fig. 61, i.e., it is found to have been compressed into the shape of a somewhat irregular truncated cone. The volume of the ideal cone indicated by the broken line in Fig. 61 might be easily calculated. An obviously far less accurate but simpler procedure is to imagine a sphere of the same volume as the truncated cone of precipitate. A circle representing the cross section of this sphere is shown in Fig. 61. Its diameter *D* is measured by means of the eyepiece micrometer and expressed in microns rather than scale divisions, the value of which depends upon the magnification used. The mass of precipitate is in direct proportion to the volume,  $v = 0.52D^3$ , and it is directly proportional to the cube of the diameter of the imagined sphere. It is obvious that a mistake of 10

per 100 in choosing the diameter of the sphere will cause an error of 30 per 100 in the derived volume and mass of the precipitate. Expressing lengths in microns, one records on one hand the diameter and the volume of the imaginary sphere, and on the other the dimensions  $B$ ,  $b$ ,  $H$ , and  $h$  of the truncated cone and its volume,  $v = 0.26 (B^2H - b^2h)$ .

**SEPARATION OF SOLUTION AND PRECIPITATE.** The tip of the micropipet is inserted in the capillary cone and advanced until its opening touches the wall of the cone at a short distance in front of the surface of the precipitate. Suction is cautiously applied by means of the plunger device so that the clear solution is taken slowly into the micropipet. Either reflected or transmitted light may be used for observation. The last portion of the solution is taken up with particular care so that the operation can be stopped as soon as air begins to enter the tip of the micropipet. The capillary cone with the precipitate is withdrawn. The micropipet is taken out of the chamber. The solution, which is no longer needed, is rejected by expelling it on a strip of filter paper. The pipet may be rinsed once with water; this step, however, may be omitted.

**WASHING THE PRECIPITATE.** Approximately 20 mL of 0.12 *M* nitric acid is taken into the micropipet from the third reagent container and transferred to the precipitate in the capillary cone. The wash liquid is delivered on the wall of the capillary cone at a short distance from the precipitate. The liquid is slowly expelled from the micropipet so that the precipitate is not stirred up. The distance from the point of the capillary cone to the meniscus of the wash liquid is measured and recorded. The wash liquid is left in contact with the precipitate for 1 minute. By the above-described technique for the separation of solution and precipitate, the wash liquid is then taken up with the micropipet and rejected. The treatment is repeated with a second portion of 20 mL of the acid. This time, a slight excess of the acid may be taken into the pipet, and the more exact measurement of the volume may be performed in the cone itself. Again, without stirring up the precipitate, one allows the precipitate to be in contact with the acid for 1 minute. The second portion of wash liquid is removed and rejected like the first.

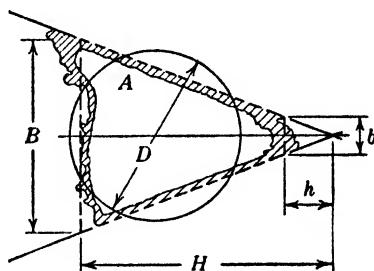


FIG. 61. Estimation of the volume of precipitates.

SEPARATION OF THE ARSENIC AND COPPER GROUPS. The sulfide precipitate is treated with 10 ml of sodium sulfide-hydroxide reagent taken from the fourth container. The reagent is slowly added without stirring. Solution of the large part of the precipitate takes place immediately.

HEATING SOLUTIONS IN THE CAPILLARY CONE. A capillary of approximately 10-cm length and 0.7- to 1-mm bore is drawn out to a fine tip at one end. A small volume of water is allowed to enter the tip, which is then sealed shut. The capillary cone with the sulfides is removed from the moist chamber, and its handle is cleaned. The cone is then introduced, handle first, into the wide capillary and made to slide down to the sealed end, Fig. 60B. Finally the open end *b* is sealed, and the capillary, with the cone inside, is placed for 5 minutes in water at 70° to 80° C.

STIRRING is usually not required. It may be nicely accomplished by withdrawing the capillary containing the cone from the bath and touching it to the vibrating part of the buzzer used for severe agitation.

PRESERVING SOLUTIONS AND PRECIPITATES IN CAPILLARY CONES. Solutions and precipitates may be preserved for several days by sealing the cone into a wide capillary as illustrated by Fig. 60B.

ESTIMATING THE QUANTITY OF BISMUTH. The capillary, Fig. 60B, is removed from the bath and cut at *a* at the end of the handle of the capillary cone. Cone and capillary are then transferred to a microcone as shown by Fig. 60A and centrifuged for the time and at the speed decided upon at the outset. The capillary cone is then transferred to the moist chamber and the volume of the precipitate is estimated and recorded. The black color of the residue of bismuth sulfide is observed against a white background by lateral illumination.

The extract containing the antimony is taken up with the micropipet and transferred to the second capillary cone. The bismuth sulfide is washed with two 10-ml portions of distilled water from the second reagent container. The first washing is combined with the extract, and the second is rejected.

PRECIPITATION OF THE SULFIDES OF THE ARSENIC GROUP. Approximately 150 ml of 0.5 *M* sulfuric acid is taken up into the micropipet from the fifth reagent container. The acid is added very slowly to the sodium sulfide extract in the second capillary cone. One must guard against too rapid an evolution of hydrogen sulfide, which would cause solution and precipitate to be expelled from the capillary cone. The mixture is stirred by plucking the copper tubing of the plunger device while adding the acid. The capillary cone is finally removed

from the moist chamber, sealed into a capillary, and heated at approximately 70° C for 10 minutes.

The precipitate is collected in the taper of the capillary cone by centrifuging. The volume of the precipitate is estimated, and the orange color of the antimony sulfide is observed by lateral illumination. Finally, the supernatant solution is removed by means of the micropipet and rejected. The precipitate need not be washed.

**STARTING A NEW SERIES OF PROCEDURES.** The reagents required for the confirmatory tests are assembled on a second carrier. Three reagent containers are required: (a) 3 *M* nitric acid, (b) quinine-iodide reagent, and (c) 12 *M* hydrochloric acid. Then the moist chamber is opened, and the capillary cones are transferred to the new carrier, which is finally substituted for the one used at the outset. In addition to the carrier, a condenser rod is placed in the moist chamber.

**SOLUTION OF THE BISMUTH SULFIDE.** The micropipet is carefully cleaned with concentrated hydrochloric acid and water. Then 10 ml of the 3 *M* nitric acid is transferred to the bismuth sulfide. The capillary cone is sealed into a capillary and then heated for 5 minutes in a bath at approximately 70° C. Without opening the wide capillary, the contents of the capillary cone are inspected under the microscope to make certain that all sulfide has been dissolved.

**EVAPORATION IN THE CAPILLARY CONE.** The end of the sealed capillary which contains the capillary cone is held in the steam escaping from a steam bath while the rest of the capillary is not heated. Thus half the solution in the capillary cone is evaporated.

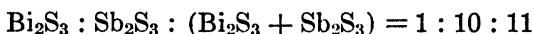
The capillary cone containing the bismuth solution is returned into the moist chamber, and 1 or 2 ml of the solution is transferred to the platform of the condenser rod and treated with a like volume of the quinine-iodide reagent. Separation of an orange or brown precipitate is observed which has a tendency to form spherulites.

**SOLUTION OF THE ANTIMONY SULFIDE AND CONFIRMATORY TEST.** The antimony sulfide is treated with 10 ml of concentrated hydrochloric acid. The capillary cone is sealed into a capillary containing some concentrated hydrochloric acid and heated at 70° C until only a white residue of sulfur remains. The capillary cone is centrifuged, and 1 or 2 ml of the clear solution is transferred to the platform of the condenser rod and treated with a like volume of the quinine-iodide reagent. Separation of a yellow precipitate confirms the presence of antimony.

**COMPILED OF RESULTS.** It is a matter of experience that equal masses of the elements arsenic, antimony, mercury, silver, lead, bismuth, and copper give sulfide precipitates of approximately identical

volumes. The volume of the sulfides of tin and cadmium shows wide variations, for these sulfides are frequently precipitated in a gelatinous state.

The test solution contained 0.1 γ of antimony and 0.01 γ of bismuth. Thus, the volumes of the sulfides should have been found to give the following ratios after division by the volume of the bismuth sulfide:



Comparison of the results will show whether or not the experimenter should base the estimation on the diameter of an imaginary sphere of precipitate. It is assumed that all the sulfides were properly flocculated, i.e., were obtained in a crystalline state.

### EXPERIMENT 58

#### Isolation of Arsenic by Distillation of the Trichloride<sup>46</sup>

An acid solution containing 0.025 γ of arsenic and 0.05 γ of antimony is precipitated with hydrogen sulfide. The volume of the precipitate is estimated, and then the precipitate is dissolved in concentrated hydrochloric acid. Solution is made complete by adding solid potassium bromate. After separation from the sulfur, the solution is treated with hydrobromic acid and phosphorous acid, first heated in a sealed capillary, and then distilled. The distillate is treated with hydrogen sulfide, and the volume of the arsenic trisulfide is estimated. The sulfide is oxidized to arsenic acid which is identified as silver arsenate. The residue of the distillation is treated with hydrogen sulfide. The volume of the antimony sulfide is estimated.

**ASSEMBLY OF APPARATUS.** A dry chamber is required in addition to a moist chamber. The moist chamber is equipped with a condenser rod and a carrier holding two capillary cones, one measuring capillary (provided that it does not seem preferable to measure the known solution in the shaft of the micropipet), and three reagent containers with: (a) known solution VI, (b) distilled water, and (c) 0.5 M nitric acid.

The dry chamber shall contain three reagent containers with: (a) 12 M hydrochloric acid, (b) 9 M hydrobromic acid, and (c) 3 M phosphorous acid. In addition to these reagent containers, a capillary cone, which fits into the opening of the distilling capillary, is placed on the carrier. The distilling capillary is held ready in a vial. It is 10 mm long in the capillary part and just wide enough in bore so that a capillary cone fits snugly into the opening, Fig. 62. The distilling capillary has a handle just like capillary cones, which may be pushed into the vaseline coating of the carrier.

**PRECIPITATION OF THE SULFIDES.** The moist chamber is placed on the stage of the microscope, and 10 ml of known solution VI is transferred to the first capillary cone and diluted with 100 ml of distilled water. The precipitation with hydrogen sulfide is carried out as described in the preceding experiment. The mixture of solution and precipitate is heated for half a minute at 60° to 80° C, and then allowed to stand at room temperature for 1 hour. After centrifuging, the volume of the sulfides of arsenic and antimony is estimated. Finally, the supernatant solution is removed and rejected. Washing of the precipitate is not necessary.

**SOLUTION OF THE SULFIDES.** The capillary cone with the precipitate is transferred to the dry chamber. The sulfides are treated with

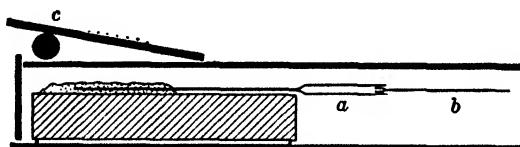


FIG. 62. Distillation from capillary cone to capillary cone, natural size.

30 ml of 12 *M* hydrochloric acid. The capillary cone is again removed from the chamber, sealed into a dry capillary, and placed in a water bath at 70° C for 15 seconds. The mixture is then thoroughly agitated by means of the buzzer. Microscopic inspection will show that only part of the precipitate has dissolved. The capillary cone is returned to the dry chamber, and its contents are treated with small portions of solid potassium bromate as follows:

**TRANSFER OF SOLIDS TO CAPILLARY CONES.** Some potassium bromate is ground to a fine powder and then sprinkled on a glass slide 2.5 cm square. By means of a short piece of glass rod, the slide is then mounted in an inclined position on top of the dry chamber, Fig. 62c. The micropipet is temporarily removed from the pipet holder, and another micropipet, the tip of which has been sealed by bringing it near a Bunsen flame, is mounted in its place. The sealed tip of the pipet is provided with a film of grease by rubbing the side of the nose with the fingertip and transferring the oil to the point of the pipet. Individual particles on the slide are focused with the microscope, picked up with the sealed tip of the pipet, and transferred to the solution in the capillary cone, which is then stirred by plucking the copper tube. The bromate is added, particle for particle, until all the sulfide precipitate is dissolved and only sulfur remains behind.

The capillary cone is centrifuged and then returned to the dry chamber. The clear solution is transferred to the capillary cone which

is held ready on the carrier for the distillation. The residue of sulfur is washed with one 40-mλ portion of 12 *M* hydrochloric acid, and the washing is transferred to the solution, which is then treated with 10 mλ of 9 *M* hydrobromic acid and 20 mλ of 3 *M* phosphorous acid. The cone is sealed into a dry capillary and placed in a bath at 80° to 90° C for 5 to 10 seconds. After cooling with tap water and centrifuging, the cone is removed from the sealed capillary and inserted in the distilling

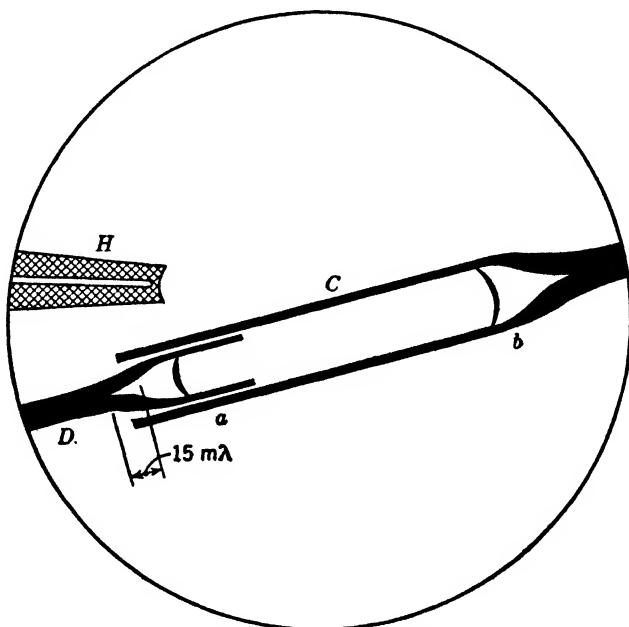


FIG. 63. Microscopic view of the distillation, approximately ten times natural size.

capillary, which is then mounted on the carrier as shown in Fig. 62. The dry chamber is again closed.

**DISTILLATION OF THE ARSENIC TRICHLORIDE.** As a rule, it will be impossible to get the whole distilling capillary into the field of vision as shown in Fig. 63. Thus, the end *a* is focused, and the mechanical stage and the eyepiece are adjusted until the point of the taper of the capillary cone coincides with a convenient scale division of the eyepiece micrometer. The scale divisions corresponding to 15 mλ and 10 mλ of liquid in the capillary cone are then recorded for immediate use. They may be quite accurately determined by observing how many divisions are required to hold the given volumes of water in a capillary cone of approximately identical shape and size. The required data may be found in the records of the preceding experiments.

Now the pipet holder is removed, and the heating element is inserted in the clamp of the manipulator. The hot point *H*, Fig. 63, of the heating element is introduced into the dry chamber and moved close to the capillary cone *D* containing the solution to be distilled. The hot point should be approximately 1 mm to one side of the capillary cone, and in the level of the distilling capillary.

The current is turned on, and the voltage is slowly stepped up while observations are made with transmitted light. Small droplets may be observed to form slowly along the walls of the distilling capillary. With a further increase of the voltage, the meniscus in the capillary cone begins to recede toward the point of the taper. This shows that fairly rapid volatilization is taking place. The rate of movement of the meniscus must be slow, and it may be adjusted by either changing the position of the hot point or by regulating the voltage supplied to the heating element. At times it happens that a gas bubble forms at the point of the taper, begins to grow, and pushes the liquid contents of the capillary cone toward the opening of the cone. If this occurs, it becomes necessary to regulate the rate of heating so as to keep this air bubble down to a small size in the point of the taper. When the receding meniscus reaches the 15-ml mark, the hot point is rapidly withdrawn from the chamber and the current is turned off. The whole length of the distilling capillary is inspected, and it will be found that the distillate collects at the end *b*, Fig. 63.

The capillary cone is withdrawn from the distilling capillary and placed on the carrier. The contents of the capillary cone are treated with 10 ml of 12 *M* hydrochloric acid, and the distillation is repeated and allowed to proceed until approximately 10 ml of residue is left in the capillary cone. Then the distillation is broken off, and the capillary cone containing the residue of the distillation is transferred to the carrier in the moist chamber. The distilling capillary is immediately centrifuged to collect all the distillate at the sealed end, and it is then cut at a distance of 3 mm from the taper. The unwanted portion of the capillary is snapped off by means of cork-tipped forceps, and the part containing the distillate is returned to the dry chamber. The distillate is immediately transferred to a capillary cone of standard dimensions by means of the micropipet.

**PRECIPITATION OF ANTIMONY SULFIDE, ESTIMATION OF QUANTITY.** The residue of the distillation is diluted with 50 ml of distilled water and treated with hydrogen sulfide. The precipitate is collected by means of the centrifuge and its volume is estimated.

**PRECIPITATION OF ARSENIC TRISULFIDE, ESTIMATION OF VOLUME.** The distillate is diluted with water to give a volume of approximately

100 ml. The mixture is treated with 40 ml of 12 *M* hydrochloric acid and then saturated with hydrogen sulfide. The precipitate has a tendency to become colloidal. Thus, the precipitation is first heated in a bath at 60° to 70° C for 30 to 45 seconds and then agitated by means of the buzzer. With lateral illumination, the contents of the sealed capillary are inspected under the microscope. If the precipitate is found to be properly flocculated, it is collected in the point of the capillary cone by centrifuging. The capillary cone is transferred to the moist chamber, and the volume of the precipitate is estimated. The yellow color of the arsenic trisulfide is observed with lateral illumination.

The supernatant solution is removed with the micropipet and rejected. The arsenic trisulfide is washed with one 100 ml portion of water.

**SOLUTION OF THE ARSENIC TRISULFIDE.** The capillary cone containing the sulfide is transferred to the carrier of the dry chamber, which has previously been cleared of other apparatus. A reagent container with 6 *M* ammonia is placed close to the capillary cone. The chamber is closed, and 10 ml of the ammonia is transferred to the arsenic trisulfide.

**EVAPORATION IN THE CAPILLARY CONE.** The cone is removed from the chamber and transferred to a microcone by means of a wide capillary open at both ends, Fig. 60A. The microcone is placed on a steam bath until the solution in the capillary cone is evaporated practically to dryness.

**CONVERSION TO SILVER ARSENATE.** The moist chamber is provided with a condenser rod and a carrier with three reagent containers. The latter hold: (a) 16 *M* nitric acid, (b) 0.5 *M* nitric acid, and (c) buffered silver nitrate solution. The capillary cone with the arsenic residue is transferred to this carrier, and the moist chamber is closed. The residue is treated with 5 ml of 16 *M* nitric acid, and the mixture is again evaporated to dryness as described above. The new residue is dissolved in 5 ml of 0.5 *M* nitric acid. After centrifuging, 1 to 2 ml of the clear solution is transferred to the platform of the condenser rod and made to evaporate by pulling the cover glass of the chamber halfway out. The chamber is again closed, and the residue is treated with 1 ml of the buffered silver nitrate solution. A brown precipitate will separate.

**SUMMARY OF RESULTS.** All estimated volumes are divided by the volume of the arsenic trisulfide. They should approximate the following ratio:



*PART III*



## QUANTITATIVE ANALYSIS

Only gravimetric and titrimetric methods of determination will be treated in some detail. Sedimentic and colorimetric procedures have been advocated in the qualitative part for the more or less crude estimation of the quantities of isolated constituents.

In practice, preference should be given to gravimetric and sedimentic methods when an object is to be analyzed which cannot be reproduced. At the conclusion of gravimetric or sedimentic procedures, the determined constituents are available in isolated form. They may be kept as evidence, and their identity, purity, and mass may be checked whenever it should become desirable to do so.

Titrimetric and colorimetric procedures frequently do not give definite proof concerning the nature of the determined matter, and the final solutions are usually more complicated in composition than the original sample. The evidence consists merely of records of instrument readings. Speed, simplicity of technique, and high accuracy under controlled conditions recommend titrimetric methods for routine work. In titrimetric microanalysis there is the additional advantage that the use of precise balances may be eliminated if the material under investigation may be measured by volume (liquid or gas) or if the microsample is obtained by aliquot partition.

The results of all quantitative determinations, no matter whether gravimetric, titrimetric, gas volumetric, sedimentic, or physicochemical methods are used, may be calculated by means of the equation:

$$P = 100f \frac{M}{S} \frac{C_1 C_2 C_3}{c_1 c_2 c_3}$$

in which the result  $P$  appears as a function of the measure  $M$  of the determined constituent (weight of precipitate, volume of standard solution, etc.) and of the mass or volume of sample  $S$ .<sup>1</sup> The letter  $f$  represents the "chemical" factor, and the product  $Mf$  has the dimension of either mass or volume of determined constituent. In gravimetric determinations  $f$  is, as a rule, the fraction of determined constituent contained in the weighing form, and the values for  $f$  may be found in tables. In titrimetric determinations,  $f$  is the product of the

<sup>1</sup> A. A. Benedetti-Pichler, *Ind. Eng. Chem., Anal. Ed.*, **8**, 373 (1936).

normality of the standard solution and the equivalent weight of the determined constituent divided by the volume of 1 liter expressed in units of the same dimension as used for  $M$ . If aliquot partitions have been employed,  $C_1$ ,  $C_2$ ,  $C_3$ ,  $c_1$ ,  $c_2$ , and  $c_3$  stand for the capacities of volumetric flasks and the volumes delivered by pipets.

Use of the equation is recommended mainly for the reason that it facilitates estimation of the effect of accidental and constant errors attached to the measured quantities  $M$ ,  $S$ ,  $C$ ,  $c$ , and  $f$  on the result  $P$  of the determination. The relative errors of the above-listed measured quantities produce identical relative errors of the result  $P$ .<sup>1</sup>

It must be emphasized that in microanalysis more attention must be given to the effects of "instrumental" errors than in macroanalysis. When determinations are carried out on large samples, the error of the results is essentially due to chemical factors such as incomplete separation, solubility of precipitates, side reactions, and indicator errors. Good analytical balances and volumetric equipment are easily operated with an accuracy which, as a rule, far surpasses the requirements of ordinary routine work. In microanalysis, however, balances, burets, pipets, and other measuring instruments are used frequently to the very limit of their capability, and a serious effort must be made to collect information on instrumental errors and their effects on the results of determinations. It is understood that the uncertainty introduced by chemical factors will remain the same in microanalysis as in macroanalysis. To attain the precision of macroanalysis, which frequently is not at all affected by the negligible shortcomings of measuring apparatus, the accidental errors of the instruments used in microanalysis must be held down to at least one-third of the uncertainty produced by chemical factors only.<sup>1</sup>

## A. GRAVIMETRIC DETERMINATIONS

### (a) CENTIGRAM AND MILLIGRAM PROCEDURES<sup>2</sup>

#### THE BALANCE

A mathematical investigation<sup>3</sup> of the relation between the precision of a weighing, the number of parts  $P$  of determined constituent in 100 parts of sample, and the conversion factor  $f$  on one hand, and of the precision of the result  $P$  on the other, has shown that the per-

<sup>2</sup> For a comprehensive treatise the reader is referred to F. Hecht and J. Donau, *Anorganische Mikrogewichtsanalyse*, Vienna, J. Springer, 1940.

<sup>3</sup> A. A. Benedetti-Pichler, *Ind. Eng. Chem., Anal. Ed.*, 11, 226 (1939).

formance of many quantitative determinations on samples of several centigrams and, at times, several milligrams mass, is possible, if a balance is available which permits reproduction of weighings within  $\pm 0.05$  mg on an average. Experience has shown<sup>1</sup> that many analytical balances of American manufacture fill this requirement, and subsequent experimental work<sup>4, 5</sup> has proved conclusively the feasibility of quantitative microdeterminations with analytical balances. To take advantage of these facts, the directions for the quantitative exercises included in this book have been organized so as to permit the use of precise analytical balances as well as of microchemical balances.

The procedure for the determination of the precision of a balance is described in Expt. 59, and it will be found that many American-made analytical balances, even inexpensive ones, are able to meet the requirement of an average deviation for a single weighing equal to or less than  $\pm 0.05$  mg.

It is suggested that the method of short swings be used in weighing. Damping devices should not be employed when a maximum of precision is desired, since the readings taken with the swinging beam are less open to accidental distortion than the fixed position of equilibrium attained with the aid of damping devices.

A description<sup>2, 6</sup> of a microchemical balance does not seem necessary. It may be simply stated that its construction follows that of the analytical balance in all essential details. It is recommended that an analytical balance and a microchemical balance as manufactured by W. H. F. Kuhlmann, Ch. Becker, P. Bunge, Sartorius, Starke u. Kammerer, and others be placed side by side, and the various parts identified and compared by following the description of analytical balances given in a textbook on quantitative analysis. The similarity cannot cause surprise when the history of the microchemical balance is recalled, which has been developed from an assay balance, constructed as a small-scale model of an analytical balance with short beam, by introducing a refined method for the polishing of knife edges. The "sensitivity" of this assay balance was guaranteed by Kuhlmann to be 0.01 mg, and its precision was probably somewhat better. The improvements incorporated in microchemical balances gave only a small increase of the reproducibility of weighings. A

<sup>1</sup> J. B. Niederl, V. Niederl, R. H. Nagel, and A. A. Benedetti-Pichler, *ibid.*, 11, 412 (1939).

<sup>2</sup> A. A. Benedetti-Pichler and R. A. Paulson, *Mikrochemie*, 27, 339 (1939).

<sup>3</sup> J. B. Niederl and V. Niederl, *Micromethods of Quantitative Organic Analysis*, 2nd ed., New York, John Wiley & Sons, 1942.

recent investigation by Corner and Hunter<sup>7</sup> showed that the average deviation of a single weighing may be expected within the limits  $\pm 2$  and  $\pm 6 \gamma$ , provided that the microchemical balance is in good condition. Errors committed in placing the rider produced an average deviation of approximately  $\pm 3 \gamma$ . Two of the 7 microchemical balances studied (two Kuhlmann, four Oertling, and one Sartorius) showed a noticeable decline of precision as a consequence of excessive wear.

The rules for the use and care of microchemical balances should be applied to any analytical balance which is to be used for work requiring high precision. It is common sense to mount any balance on a sturdy table so as to protect the instrument against shock and excessive vibrations. The table should be in a location where the balance will not be exposed to draft or direct sunlight; the proximity of heating devices, radiators, steam pipes, and pipes which could cause a cooling effect has to be avoided. The balance room should be reasonably free from abrasive dust, and the temperature of the room should not undergo frequent or rapid changes.<sup>8, 9</sup> Instructions for the setting up of balances are furnished by the manufacturers.

The cleaning and adjusting<sup>2, 6, 10</sup> should always be carried out either by or under the supervision of an experienced worker. First the balance case is carefully cleaned outside and inside with moist gauze. Then the parts of the balance are cleaned from dust by means of a soft brush. The use of rubber gloves or finger cots is highly recommended, for the gold or platinum plated parts should not get in contact with the skin. The two most important tasks are left to the last: the cleaning of the arresting contacts and of the bearings. These are freed from dust and grease by rubbing with small pieces of old linen which has been carefully washed with soap and distilled water and dried between layers of clean filter paper. The linen pieces, which must be frequently changed, are applied by means of ivory-tipped forceps and toothpicks. Finally, a camel's-hair brush, which has been washed in acetone and dried, is used to remove all fibers left on the arresting contacts, the bearings, the pointer needle, and the rider scale of the beam. A thorough cleaning of the balance should be performed only once or twice a year, depending upon the

<sup>7</sup> Mary Corner and H. Hunter, *Analyst*, **66**, 149 (1941).

<sup>8</sup> On the mounting of microchemical balances under adverse conditions see W. R. Kirner, *Ind. Eng. Chem., Anal. Ed.*, **9**, 300 (1937).

<sup>9</sup> E. Schwarz-Bergkampf, *Z. anal. Chem.*, **69**, 321 (1926).

<sup>10</sup> F. Emich and F. Schneider, *Microchemical Laboratory Manual*, New York, John Wiley & Sons, 1932.

conditions in the balance room. It should be understood, however, that chemicals accidentally spilled inside the balance case must be immediately and thoroughly removed.

### WEIGHING WITH MICROCHEMICAL AND ANALYTICAL BALANCES

With European balances the whole arresting mechanism is operated by means of a single milled head or crank in such a way that automatically *first* the pans are released and then the beam. Likewise the pans are arrested *after* the beam. This order has been chosen for the protection of the knife edges from unnecessary jars and jolts which would ensue if the pan arrests operated while the central and terminal bearings of the beam were engaged. With many analytical balances of American origin, the mechanism of the pan arrest is operated independently from that of the beam arrest. In order to get the best results with these balances, it is necessary to *avoid releasing or arresting the pans while the beam is released*. This advice is contrary to the usual practice, but it should be *strictly* followed.

Counterbalancing is performed in the usual manner. One should take care, however, to touch the pans or objects thereon only when the balance is completely arrested. In the final adjustment with the use of the rider, it is necessary to arrest the beam before moving the rider, but it is superfluous to arrest the pans. It is understood that, in counterbalancing, the beam should not be completely released until the equilibrium is so far established that the swing of the beam remains within the pointer scale. With microchemical balances this will not happen before the masses on both sides are made equal to the nearest tenth of a milligram, i.e., until the rider is not more than one notch from its final position. Finally, before proceeding to the actual weighing, one must check up whether the pans remain perpendicular and motionless when released, and whether the rider is situated in a definite, easily reproducible position. A rearrangement of the objects on the pan will correct the position of the pans; repeated operation of the pan arrests will stop the swinging motion. As to the position of the rider, a conscientious effort must be made to meet the following requirements: the rider should occupy the precise place indicated by the selected division of the rider scale; it should be exactly upright; and its plane should be perpendicular to the rider scale as indicated in Fig. 64.\*

The weighing is performed by the method of short swings. The beam is slowly released so as to cause a swing of an amplitude not greater than five nor less than three divisions of the pointer scale. If the swing is too wide, the beam may be arrested and released more slowly, or one may wait until the width of the swing has sufficiently decreased. If the swing is too short, careful opening and closing of a side door may help with microchemical balances. With analytical balances, opening of a door and a fanning motion with the hand may be necessary. No matter how the desired amplitude is obtained, it

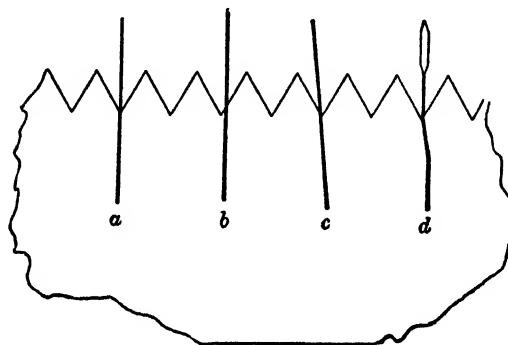


FIG. 64. Positions of the rider: *a*, correct position; *b*, rider placed too far to the right-hand side; *c*, rider inclined toward the left; *d*, plane of rider not perpendicular to that of the rider scale.

is advisable to disregard the first two full swings. The following three consecutive points of inflection are read and used for the calculation of the deflection.

The deflection<sup>3</sup> (synonymous with "deflection difference"<sup>10</sup> and "deflection sum"<sup>6</sup>) is the value of the other point of reversal of an ideal swing which has zero for one point of inflection. Herein, the center of the pointer scale is assigned the value zero; the right side is counted positive, and the left side negative, Fig. 65. The tenths of the scale divisions are estimated when the points of inflection are read, and in order to avoid the use of superfluous decimal points, the tenth of a scale division is taken for the unit ("deflection unit"<sup>6</sup>). Thus, the customary pointer scale comprises 120 positive units from the center to the right and the same number of negative units from the center to the left.

In Fig. 65 three consecutive positions 1, 2, and 3 of the pointer are indicated. They correspond to the following points of inflection:

## SWING TO THE RIGHT

First	+36
Second	
Third	+34
Average	<u>+35</u>

## SWING TO THE LEFT

-20

The amplitude of the corresponding ideal (frictionless) swing is estimated by averaging the two points of inflection observed on one side

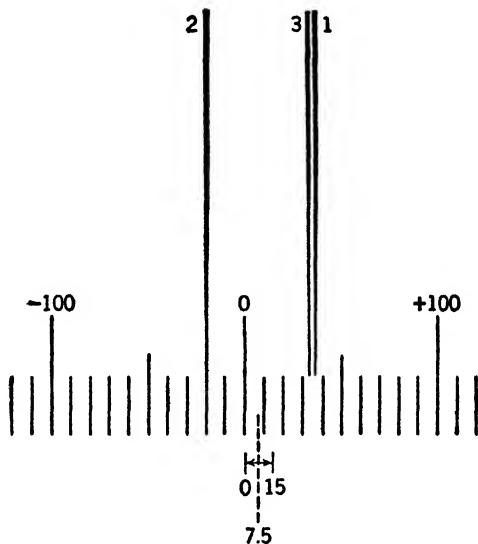


FIG 65. Pointer scale, determination of the deflection.

of the swing. The ideal swing which corresponds to the observed actual swing would have +35 and -20 for points of inflection and +7.5 as center. If one imagines that the amplitude of the ideal swing continuously decreases, there must be a time when 0 and +15 are observed as points of inflection; at this time the deflection is identical with the value of the point of inflection at the right-hand side, +15. Mathematically the deflection is obtained by algebraic addition of the points of inflection of the ideal swing:  $+35 + (-20) = +15$ .

If the following points of inflection are observed in the order listed: -75, -21, and -73, the deflection is -95, and when the swing finally ceases, the pointer should stop at -47.5.

It is obvious that the evaluation in terms of mass of an observed deflection requires the knowledge of the **sensitivity** of the balance,

which is defined as the mass required to change the deflection for one unit (0.1 division) of the pointer scale.

Analytical balances differ widely with respect to the arrangement of the rider scale. Many have the zero mark at the center of the beam and indicate five or ten milligram divisions to the right and the left of the beam. Before any work is begun, it is suggested that a rider of 5-mg mass be placed on the first division or notch on the left-hand side of the beam, which may be marked "5" or "10," and to bring the balance to equilibrium with the use of the adjustment screws usually located at both ends of the beam. It is understood that this adjustment should be performed by an experienced worker. When a balance thus adjusted is being used it must be kept in mind that transference of the rider to the center division (marked zero) is equivalent to placing a 5-mg piece on the right-hand pan. If the rider is placed on the last division on the right-hand side of the beam, it has an effect equal to that of a 10-mg piece on the right-hand pan. All the values indicated on the rider scale change accordingly.

### EXPERIMENT 59

#### Determination of the Precision and Sensitivity of the Balance

The precision of a balance must be determined under conditions which closely approximate those which prevail when the balance is used in quantitative determinations.<sup>1</sup> The following directions are an attempt to meet this requirement. In addition they may be considered a code of proper behavior during the performance of precise weighings.

1. The balance case is opened at the beginning of every working period, and, by means of a clean camel's-hair brush, dust particles visible on the pans and on the base plate of the balance are removed. The doors of the case are left open, and the experimenter leaves the position in front of the balance and does not resume work there before sufficient time has elapsed to ensure that the interior of the balance case has acquired the temperature of the room and that any temperature gradients set up in the instrument have disappeared. Five minutes will suffice for an analytical balance, but with microchemical balances a waiting period of 15 to 20 minutes may be required.

For best results with microchemical balances, it is advisable to place a thermometer inside the balance case and to watch it while working with the balance. As soon as the temperature in the balance

case has risen  $0.3^{\circ}$  C, the work should be interrupted until the original temperature is again established. Furthermore, it is good practice to release and arrest the microchemical balance five times at the start of every working period so as to make certain that strains in the beam which may have developed during the interim<sup>9</sup> are released.

As soon as work is once started, cleaning of the balance proper should not be repeated. The doors of the balance should remain slightly ajar during the whole working period, being closed only for the performance of weighings. The experimenter must never linger in front of the balance when his presence is no longer required. This precaution is necessary with microchemical balances, the temperature equilibrium of which may be upset by the heat radiation from the body of the experimenter if he stays near the balance for more than 3 minutes at a time.

2. One 10-gram weight is placed on the center of the left-hand pan, and another 10-gram piece on the center of the right-hand pan. The doors of the balance are closed. (Ten grams may be considered an average load in the performance of microchemical determinations.)

3. The rider is placed in the position zero, and a test is made to determine whether the equilibrium of the balance is sufficiently established. If necessary, the rider is moved, and after the proper location of the rider has been found, it is made certain that the rider assumes the correct position. When using a microchemical balance, the doors are then opened and the observer leaves the balance for 3 minutes. Such a period of rest before the weighing proper should be allowed the microchemical balance whenever the counterbalancing requires more than 1 minute. These periods of rest are frequently mentioned on the pages to follow, and it should be understood that they may be eliminated when working with analytical balances which are little affected by small temperature changes.

4. The doors of the balance case are closed, and a waiting period of 20 seconds is observed to give air currents set up in the case by the motion of the doors time to subside. The balance is then slowly released, care being taken that a swinging motion of the pans is stopped before the beam is released. The first two complete swings are disregarded, and the following three positions of inflection are observed and recorded. To avoid bias, the record is immediately covered with a sheet of paper. The deflection is calculated after *all* weighings for the determination of precision have been performed.

The balance is arrested with proper care. The doors of the case are opened, and the balance is left alone for 3 minutes. This period of rest is omitted when working with an analytical balance.

5. The doors of the balance case are closed, and the rider is lifted off the beam, moved sideways with the rider-carrier, and then returned to its former position as exactly as possible. The balance is released. Two full swings are disregarded, and then three consecutive points of inflection are read and recorded. The record is quickly covered and the balance is arrested. A microchemical balance is again allowed a 3-minute period of rest with doors ajar.

6. Eight more weighings are performed as directed under 5.

7. A new series of weighings is started for the determination of the sensitivity of the balance. The balance case is closed, and the rider is moved from its position for a distance corresponding to 1 mg when working with an analytical balance, or for a distance corresponding to 0.1 mg (1 notch) when working with a microchemical balance. Three more weighings are performed as directed under 5.

8. After the balance has been arrested finally, the rider is transferred to the position zero. The balance case is opened, and the weights are removed from the pans. The balance is left with the doors of the case slightly ajar.

9. Of both series of weighings, the deflections and their arithmetical means are calculated. The first series of ten weighings serves for the calculation of the average deviation of a single weighing: the deviations of the individually observed deflections from the calculated mean are listed in an orderly manner. The arithmetical mean of the deviations, which are added without considering their sign, represents the average deviation of a single weighing in units of the pointer scale.

#### CALCULATION OF THE PRECISION OF A WEIGHING IN UNITS OF THE POINTER SCALE

<i>Rider at 0.1 mg, (1st series)</i>	<i>Deviations from mean calculated</i>	<i>Rider at 1.1 mg, (2nd series)</i>
deflections observed	deflections observed	
+67	+4	-15
+62	-1	-14
+61	-2	-16
+64	+1	
+62	-1	
+66	+3	
+58	-5	
+63	0	
+64	+1	
+63	0	
<hr/>		Mean -15
+63 Mean	$\pm 1.8$ Average deviation of single observation	

10. *Sensitivity. Precision of a Single Weighing.* Division of the change of weight in milligrams (caused by the displacement of the

rider between the two series of weighings) by the difference of the mean deflections of the two series gives the value of one unit of the pointer scale in milligrams:

## MEAN DEFLECTIONS

Rider at 0.1 mg .....	+63
Rider at 1.1 mg .....	-15

Algebraic differences: 1.0 mg .. .... 78 units of the pointer scale

$$\text{Thus, 1 unit of the pointer scale} = \frac{1.0}{78} = 0.013 \text{ mg.}$$

The *sensitivity* of the balance appears as the value of 1 unit of the pointer scale expressed in milligrams. It may be repeated that 1 unit corresponds to 0.1 division of the pointer scale.

The *precision* of a single weighing is finally obtained in terms of absolute mass by multiplication of the average deviation with the sensitivity: Average deviation of a single weighing,

$$a_s = \pm 1.8 \times 0.013 = \pm 0.023 \text{ mg.}$$

The average deviation of a single weighing, i.e., the precision of the balance, indicates the limitations of the instrument and permits the calculation of the smallest weight of material which can be used for quantitative determinations without danger of introducing significant weighing errors.<sup>3</sup> A future weighing will hardly ever show a deviation exceeding a value four times the determined average deviation, provided that all weighings are carried out with the same exactness and care, that the precision of the balance is not impaired by abuse, and that the condition of the balance room remains up to the standard established at the time of the determination of the precision. As to microchemical balances, it must be pointed out that the precision found as described above holds only for weighings performed in rather rapid succession. If hours are allowed to lapse between the consecutive weighings, a considerably poorer precision would be found if the temperature of the balance room is not kept constant within 1 degree Centigrade.<sup>9</sup> Where such ideal conditions cannot be established, it is advisable to use 2- to 5-mg samples even though the calculations based on the determined precision would indicate the feasibility of work with far smaller quantities.

As to the sensitivity, it will be noted that for many microchemical balances the value of 1 unit of the pointer scale is closely equal to 0.001 mg, a circumstance which facilitates the evaluation of the deflection in terms of absolute mass. The sensitivity of these balances is intentionally adjusted to this end. The beam of the balances is of

sufficient rigidity to assure constancy of the sensitivity up to the maximum load of usually 20 grams. The use of the deflection for the evaluation of weighings by the method of short swings, and the intentional adjustment of the sensitivity for the simplification of the calculation, seem to have originated with Paul Bunge and W. H. F. Kuhlmann.<sup>3, 11</sup>

## EXPERIMENT 60

### Calibration of Weights

The custom of using permanent tares for the counterbalancing of apparatus makes it unnecessary to calibrate more than the 10-mg and 20-mg pieces. A 5-mg piece is not required, since 10 mg may be added by means of the rider. Most of the weighings are carried out with the use of permanent tares and the rider only, and for this reason the mass of the rider is taken as standard and the weights are calibrated relatively to the mass of the rider. The method of substitution is used. Pieces taken from another set of weights serve as auxiliary masses. The procedure follows:

1. The 10- and the 20-mg pieces, which are to be calibrated, are inspected for cleanliness and freedom from corrosion. Dust is removed by means of a camel's-hair brush.

2. The 10-mg auxiliary piece is placed on the left-hand pan, and the rider is brought as correctly as possible in the position 10 mg. The balance case is closed, and the deflection  $d_1$  is determined. After arresting the balance, the 10-mg piece which is to be calibrated is placed on the right-hand pan, and the rider is brought exactly to the position zero. The balance case is closed, and the deflection  $d_2$  is determined. It is understood that a microchemical balance should be allowed a period of rest (3 minutes) before this second weighing is performed. The mass of the 10-mg piece is calculated from the equation

$$10.000 + d_1s = (10\text{-mg piece}) + d_2s$$

in which  $s$  denotes the value of 1 unit of the pointer scale in milligrams (sensitivity). It is obvious that the deflections must be entered in the equation with their proper signs.

The sensitivity of many analytical balances varies considerably with the load on the pans. It will be advisable, when working with an analytical balance, to determine the sensitivity for very small loads

<sup>11</sup> W. Ostwald and R. Luther, *Hand- und Hülfsbuch zur Ausführung physiko-chemischer Messungen*, 2nd ed., Leipzig, W. Engelmann, 1902, pp. 49 and 143.

by finally shifting the rider from the position zero to the position 1 mg and determining the deflection  $d_3$ .

$$s_{\text{very small load}} = \frac{1}{d_2 - d_3} \text{ mg per unit of the scale}$$

3. A 20-mg auxiliary mass is substituted for the 10-mg auxiliary mass on the left-hand pan and weighed (a) by means of the calibrated 10-mg piece already on the right-hand pan and the rider, and (b) by means of the 20-mg piece which is to be calibrated. The calculation follows the principle outlined above.

The calibrated weights are best kept inside the balance case in a small Petri dish the bottom of which is lined with clean silk or velvet.

## EXPERIMENT 61

### Residue Determination: Determination of Sodium in Sodium Oxalate<sup>5, 12</sup>

$\text{Na}_2\text{C}_2\text{O}_4 : P = 34.32$  per cent sodium.

$\text{Na}_2\text{SO}_4 : 100f = 32.38$  per cent sodium.

$\text{Log } 100f = 1.5103$ .

Mass of sample,  $S = 400a_s$ <sup>3</sup>

Weighing form ignited at approximately 700° C.

#### Required:

Platinum crucible, 1-ml capacity, and cover.

Forceps with platinum tips.

Aluminum bar for evaporation.

Evaporating dish or crucible cover of fused quartz, approximately 8-cm. diameter.

Small bell jar.

Sodium oxalate, Sørensen, tested purity.

Sulfuric acid, concentrated, pure (Appendix).

The sample is weighed in a small platinum crucible, then treated with concentrated sulfuric acid and ignited. The sodium content is calculated from the weight of the sodium sulfate formed.

The platinum crucible used in this experiment has a height of 16 mm and is of slightly conical shape with a diameter of 10 mm at the bottom and approximately 13 mm at the top. The weight of crucible and closely fitting lid is in the neighborhood of 2 grams. A suitable tare is best prepared from thick platinum or gold wire; by means of an analytical balance, the length of the wire is adjusted so that the

<sup>12</sup> On the performance of residue determinations with the use of electrically heated muffles see Cl. J. Rodden, *Mikrochemie*, 18, 97 (1935), and A. R. Norton, G. L. Royer, and R. Koegel, *Ind. Eng. Chem., Anal. Ed.*, 12, 121 (1940).

crucible and cover are 0.2 to 0.5 mg heavier than the wire. The final adjustment should be made with a file or sandpaper. The wire is finally bent into a spiral with handle, Fig. 67B, or into a shape indicative of a tetrahedron, Fig. 67C. If weights are used in the place of such a permanent tare, it is best to leave the weights on the pan of the balance until the last weighing of the determination has been performed; thus, it is made reasonably certain that the same pieces of weight are used in all weighings belonging to one determination.

When counterbalancing 2 grams of platinum with brass weights, a change of 5 per cent in the density of air would affect the weighings to the amount of 9 γ, because of the difference in buoyancy. It is not to be expected, however, that such a considerable change in the density of air will take place within 2 hours, which is approximately the time elapsing between the first and the last weighing of the contemplated determination.

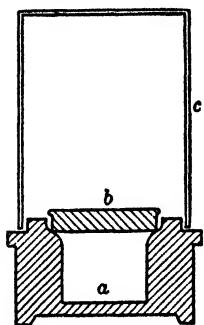


FIG. 66. Micro-desiccator, about half natural size.  
Wm. Saschek, *Ind. Eng. Chem., Anal. Ed.*, 9, 491 (1937).

CLEANING OF PLATINUM CRUCIBLES. Crucible and cover are cleaned under a stream of tap water by means of a small brush or a wad of cotton on a toothpick. This is followed by rinsing with distilled water, and then crucible and cover are placed on a disk of clean filter paper.

The cooling block *b* is taken out of the desiccator, Fig. 66. The surface of the block is cleaned with a camel's-hair brush, and the block is then returned into the desiccator. The platinum crucible is picked up by means of the platinum-tipped forceps, held in the top portion of a non-luminous Bunsen flame, and rotated so that all parts of it are heated at one time to incandescence. The whole operation does not require more than 30 seconds. After withdrawal from the flame, the crucible is allowed to cool for a few seconds and then is placed on the cooling block in the desiccator. The lid is briefly ignited and then placed on the crucible, whereupon the desiccator is closed.

TRANSFER OF MICROCRUCIBLE TO BALANCE. After 10 minutes the crucible will have attained room temperature. The desiccator is placed near the balance and opened. The cooling block carrying the crucible is taken with the fingers and placed inside the balance case near the left-hand pan. The crucible is then grasped with clean forceps just below the lid and transferred to the center of the left-hand pan. When a microchemical balance is employed, these operations are performed with the left hand inserted through the side

door of the balance case. The cooling block is immediately returned to the desiccator.

**COUNTERBALANCING.** Crucible and lid are counterbalanced by means of the permanent tare and the rider. The tare is placed on the right-hand pan, and the rider is left in the position zero. The case is closed and the balance is slowly released until a movement of the pointer indicates which side of the balance is heavier. Then the balance is immediately arrested without ever having been completely released.

If the tare is properly adjusted, the crucible should be heavier than the tare. The rider is transferred to the center of the beam, and again the balance is partially released for a trial. Provided that the tare is properly adjusted, the right-hand side will now be found to be the heavier. Thus, the rider is placed halfway between 5 and 0, i.e., at 2.5, and the trial is repeated. Once again the crucible should be too light, and the rider is now transferred to a position halfway between 2.5 and 0 (1.2), etc. When it is finally found that the left-hand side is too light with the rider at 0.6 and too heavy with the rider at 0.3, the rider is transferred to 0.4 or 0.5, and the balance is completely released for a trial, since the counterbalancing must now be sufficiently close to keep the swing of the pointer within the range of the pointer scale.

It is obvious that the above instructions apply strictly to microchemical balances only. The principle of using for the next trial the halfway point between two former rider positions should be retained when working with an analytical balance, but the *milligram divisions* of the rider scale *should be used exclusively*. Use of the subdivisions of the rider scale in attempts to establish "better" equilibrium is plain waste of time in weighing with analytical balances.

**WEIGHING.** The preliminary trials have shown what position of the rider should be chosen for the weighing. The rider is placed in the selected position as exactly as possible. The balance case is opened and the experimenter leaves his position in front of the balance for 3 to 5 minutes. This period of rest is omitted when working with an analytical balance, and even with microchemical balances it may be omitted if the counterbalancing does not require more than a few seconds.

After the balance case has been closed, the balance is slowly released. Two full swings are disregarded, and then three consecutive points of inflection are read. The balance is arrested, and the doors of the case are opened.

In the calculation of the weight only the position of the rider and the observed deflection are considered.

**TAKING THE SAMPLE.** Sodium oxalate of tested purity, as suggested by Sørensen for the standardization of permanganate solutions, is used for the experiment. Two to five milligrams of it is taken when working with a microchemical balance. If an analytical balance of the precision  $a_s$  is used, approximately  $400a_s$  mg of sodium oxalate is taken.<sup>8, 5</sup>

The cooling block of the desiccator is placed close to the left-hand pan of the balance. The crucible is grasped just below the lid by means of forceps and transferred to the block. The block with the crucible is then placed on a clean sheet of writing paper. The lid of the crucible is picked up with forceps and laid on the cooling block. The crucible is placed on the paper. The estimated quantity of sodium oxalate is now transferred to the platinum crucible by means of a penknife or a suitable spatula. The beginner finds it difficult to visualize the volume of the sample of the desired mass. It is obvious that the density of the salt may be used for an approximate calculation of the volume.

Before returning the crucible to the pan of the balance, one knocks lightly at the outside wall with the clean spatula or penknife. This causes any salt which might accidentally adhere to the outside to fall off. The crucible is then grasped with forceps, transferred to the cooling block, covered with the lid, transferred with the block into the balance case, and placed on the pan as described above. The cooling block is returned to the desiccator immediately.

Counterbalancing is started by adding weight equal to the desired weight of sample to the right-hand side of the balance. A few quick tests will show whether or not the mass of the sodium oxalate deviates considerably (more than  $\pm 20$  per cent) from the desired figure. If necessary, the crucible is returned to the sheet of paper, which is first carefully wiped to remove any salt from its surface. If the hands have been freshly washed the skin along the side of the palm is very suitable for such cleaning operations, since it does not leave fibers behind. Adding more oxalate is simple, but if too much has been taken it will be best to reject the whole contents of the crucible and to introduce another, smaller sample.

It may be necessary to allow the balance a period of rest before the weighing proper is performed. After weighing, the covered crucible is returned to the desiccator by means of the usual routine with the cooling block. The tare remains on the right-hand pan, provided

that the balance is not required for other work. The doors of the case are left slightly ajar.

**TREATMENT WITH SULFURIC ACID.** The following procedure has the purpose of preventing losses caused by the creeping of the sulfuric acid during its evaporation.

A strip of aluminum sheet 24 cm long, 3 cm wide, and 5 mm thick is provided with three circular holes 2 cm apart, the first of which is 4 cm from one end of the strip as shown in Fig. 67D. The holes are

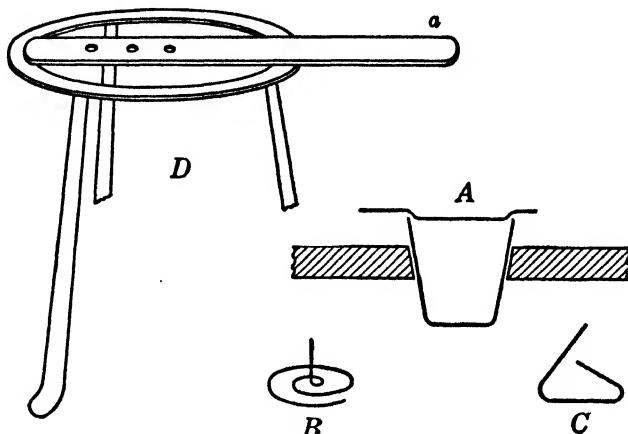


FIG. 67. Residue determination. *A*, 1-ml platinum crucible in an opening of the heating bar; *B* and *C*, tares; *D*, heating bar placed on tripod.

bored in a slightly conical fashion and their surfaces are carefully polished so that the platinum crucible fits snugly into the holes in a position indicated by Fig. 67A. The aluminum bar is laid across the ring of a tripod so that the end *a*, Fig. 67D, projects as far as possible beyond the ring support.

The crucible with the sodium oxalate is transferred in the desiccator to the heating device. The desiccator is opened, and the lid of the crucible is lifted off and placed upside down beside the crucible on the cooling block. The crucible is placed in one of the holes of the heating bar, which is able to accommodate three crucibles at a time. A small volume of pure concentrated sulfuric acid (1  $\lambda$  for every milligram of oxalate, but not less than 5  $\lambda$ ) is added by means of a capillary pipet. A spot on the inside of the crucible is selected which is obviously free from salt and close to the bottom. There, the wall is touched with the tip of the pipet, and the acid is deposited by gentle blowing with the mouth. The crucible is covered with the lid, and the heating of the end *a* of the aluminum bar is started. If a

short thermometer is placed on the bar so that the bulb is close to the crucible, the rate of heating may be controlled better. Using the nearly full, non-luminous, but silent flame of a strong Bunsen burner, a temperature of 250° C at the crucible will be reached within 12 or 15 minutes. Fumes of sulfur trioxide will begin to escape from the crucible at approximately 180° C, and when 250° C is reached, the sulfuric acid is usually completely expelled. If not, the temperature is held at 250° C until the liberation of fumes ceases. Then the crucible is grasped with forceps just below the lid and placed in an evaporating dish of fused quartz, which is held close to the heating bar. The dish with the crucible is placed on a triangle supported by a ring stand, and the quartz dish is heated with the full flame of a strong Bunsen burner. The highest obtainable temperature is held for 5 minutes.

**FUMING WITH AMMONIA.** The conversion of the bisulfate contained in the residue to sulfate is accomplished as follows. The platinum crucible is transferred to a thick glass plate, approximately 4 cm square, which is then placed on a large glass plate or directly on the table top. The lid of the crucible is removed and placed upside down next to the crucible on the small glass plate. A crucible or small evaporating dish containing a few milliliters of concentrated ammonia is placed close to the platinum crucible, and the whole is covered with a small bell jar and allowed to stand for 15 minutes. The platinum crucible is then returned to the aluminum heating bar, covered with the lid, and heated as already described. It is finally transferred into the dish of fused quartz and ignited as before.

When the quartz dish has cooled to approximately 200° C, the crucible is transferred to the cooling block and allowed to stand in the desiccator for 10 minutes. The platinum crucible is then transferred to the balance and weighed. Treatment with ammonia, heating, ignition, and weighing are repeated until the weight of the crucible remains constant within  $\pm 3a_s$  mg. As a rule, one repetition of the procedure is sufficient.

**INSPECTION OF THE RESIDUE.** The determination gives theoretical results, if creeping does not occur during the treatment with sulfuric acid. If, however, the final inspection of the interior of the crucible shows a crust of salt reaching up to the rim of the crucible, an incorrectly high result may be expected. Under such conditions the temperature obtained on the upper part of the crucible during ignition on the quartz support is frequently not sufficient for the decomposition of all bisulfate. If the residue is dissolved in a drop of water and the

solution is tested with blue litmus paper, the color change to red will show the presence of bisulfate.

**RECORD AND CALCULATION.** It is suggested once and for all to record all weighings belonging to one determination in the order in which they are performed in one perpendicular column. Four-place logarithms usually suffice for the calculation of results. The following example of a residue determination with an analytical balance ( $a_s = \pm 0.015$  mg) may serve as illustration. The words set in italics are inserted for the purpose of explanation and should be omitted in the records of an experienced worker.

#### DETERMINATION OF SODIUM IN SODIUM OXALATE

Weightings	Calculation
0.31	<i>log sulfate</i> 8156
6.45 —— 6.14 mg $\text{Na}_2\text{C}_2\text{O}_4$	<i>log factor</i> 5103
6.85 —— 6.54 mg $\text{Na}_2\text{SO}_4$	3259
	<i>log oxalate</i> 7882
	<hr/> 5377
	34.5 per cent sodium

The results of the practice determination should agree with the calculated sodium content of sodium oxalate within the limits  $\pm 0.25$  per cent sodium.

#### TREATMENT OF APPARATUS PREVIOUS TO WEIGHING<sup>13</sup>

Substances to be weighed are practically never placed directly upon the pan of the balance. As a rule they are transferred to the balance in apparatus which frequently has a mass 1000 times greater than the substance itself. The weight of the substance proper is then obtained as the difference between the weight of apparatus plus substance and the empty apparatus, i.e., the small mass of the substance is frequently obtained as the difference of two considerably larger masses. This procedure, which in principle is adverse to the maintenance of high precision, is permissible because of the extraordinary reliability of the balances. It is imperative, of course, to keep the mass of the apparatus constant within at least the same limits which hold for the reproducibility of the weighings. This is accomplished by meticulously cleaning the apparatus at the start of the experiment, preventing extraneous matter from entering the apparatus, and, if

<sup>13</sup> On drying and the weighing of hygroscopic substances see H. K. Alber, *Mikrochemie*, 25, 47, 167 (1938), D. F. Hayman, *Ind. Eng. Chem., Anal. Ed.*, 10, 55 (1938), and R. T. Milner and Mildred S. Sherman, *ibid.*, 8, 427 (1936).

necessary, cleaning its outside again before the second weighing is performed.

The cleaning operations remove dirt and dust. For an exact reproduction of the weight, it is also necessary that the apparatus have the temperature of the balance room and that the surface film of adsorbed moisture be kept constant. Both these requirements can be satisfied by allowing the apparatus to stand for a reasonable length of time in contact with the atmosphere of the balance room. Tongues or forceps with long handles should be used in the transfer of the apparatus to and from the pan of the balance so as to avoid transmitting the warmth of the hand to either the apparatus or the interior of the balance case.

Special cleaning operations are omitted after ignition of the apparatus as practiced in Expt. 61. It may be pointed out, however, that ignition at temperatures close to or above 1000° C should be avoided whenever possible, since apparatus of porcelain, quartz, and platinum undergoes changes of mass of a magnitude significant in microanalysis.

Apparatus which is not ignited is prepared for weighing by wiping it first with a moist cloth and then with a reasonably dry chamois.<sup>11, 14</sup> The moist cloth assists in the cooling of apparatus taken from a drying oven or block, and it helps to restore the film of adsorbed moisture on the surface of the apparatus. Naturally, it is not permissible to touch the apparatus with the fingers after cleaning.

The cloth and the chamois used must be clean, and they must consist of a material which does not give off fibers. Pieces approximately 12 cm square of a good white flannel and of genuine chamois leather are recommended. Before touching them, the hands must be carefully washed. When new, and whenever they lose the appearance of perfect cleanliness, they are thoroughly washed with lukewarm water, soap, and a few drops of ammonia. The rinsing is performed first with tap water and finally twice with distilled water. For drying, the pieces of chamois are spread between layers of clean filter paper. The flannel is pressed between the fingers to remove the excess of moisture so that no drops of water are left behind when it is used to wipe a piece of glass tubing. It is then placed in a jar with screw cap to protect it from dust and to prevent the rapid loss of moisture. The chamois is kept in a similar jar. A certain amount of humidity is imparted to the chamois by placing one of the moist flannels for 10

<sup>14</sup> F. Pregl, *Die quantitative organische Mikroanalyse*, 1st ed., Berlin, J. Springer, 1917.

minutes in the jar with the chamois. The use of perfectly dry cloth must be avoided, since the wiped glass surfaces would become strongly charged with electricity.

If a reasonable amount of humidity is present in the air, the electric charges are dispelled within a few minutes. In dry weather, however, they may be retained for hours on the surfaces of quartz and chemically resistant glasses which contain a high percentage of silica. Soft glass and Fiolax glass, made by Schott u. Gen. in Jena, Germany, give little trouble, and the Fiolax glass has a high resistance to chemical attack. The wiping of apparatus of clear fused quartz, Pyrex glass, or Geräteglass of Schott u. Gen. should either be avoided or followed by special treatment for the dispelling of the created electric charge.

All the methods for dispelling the electric charge on glass apparatus are based on exposure of the charged surfaces to a highly ionized atmosphere. If otherwise permissible, the apparatus is grasped with forceps and drawn through a non-luminous Bunsen flame with reasonable speed. Exposure to the silent discharge of a high-frequency vacuum tester is recommended by Van Straaten and Ehret.<sup>15</sup> Rodden<sup>16</sup> found irradiation with the light of a Hanovia Alpine Sunlamp or a G. E. Lab-Arc satisfactory. The wiped object is placed for 10 minutes at a distance of approximately 2 feet from the source of light. Finally, one may ground the aluminum base of the desiccator and place within the desiccator, next to the apparatus in question, some highly radioactive material. Spent emanation tubes have been repeatedly recommended for this purpose. Of course, the hazard to health must be given proper consideration, if gamma radiations are obtained. Keeping a piece of pitchblende in the balance case is quite useless, since the radiation is too weak to produce sufficient ionization.

It must be emphasized that electric charges may seriously affect the weighings. The attraction forces set up between the charged piece of apparatus and its surroundings may cause errors of the magnitude of 1 mg. In addition, it must be remembered that various parts of the balance are electrically insulated from one another by means of agate bearings and agate arresting contacts for beam, stirrups, and pans. Induction and accidental grounding may lead to the formation of electric charges on parts of the balance which may persist for a long time after the removal of the charged apparatus from the balance case. The author was once shown a microchemical balance which had

<sup>15</sup> F. W. Van Straaten and W. F. Ehret, *Ind. Eng. Chem., Anal. Ed.*, **9**, 443 (1937).

<sup>16</sup> Cl. J. Rodden, *Ind. Eng. Chem., Anal. Ed.*, **12**, 693 (1940).

temporarily assumed labile equilibrium. The phenomenon could be explained by the formation of electric double layers on the left pan and the brass part below it, the two being separated by an agate arresting contact.

Obviously, the weighing of apparatus carrying electric charges must not be attempted. For the sake of the reliability of the balance, such apparatus should never be introduced into the balance case. If much work is done in a very dry atmosphere, it is advisable to have ready a leaf electroscope or a silk fiber <sup>6</sup> suspended from a simple stand, and to test each apparatus for electric charges before bringing it into the balance case.

## EXPERIMENT 62

### Wiping of Apparatus Previous to Weighing

A microbeaker, Fig. 69A, is rinsed with tap water and distilled water, and heated in a drying oven for 10 minutes at 120° C. Its outside is wiped with a clean cloth before being placed in the oven. The doors of the balance case are opened halfway to assure uniformity of temperature inside and outside the case, and a desiccator, Fig. 66, is held ready. The cooling block is removed from the desiccator, and the surface of the cavity is wiped with a clean chamois, which should be reserved for this purpose. Any fibers or dust particles may finally be removed by blowing clean air over the cavity.

After removing the hot beaker from the oven, one grasps it by means of the moist flannel. Care must be taken that no moisture or fibers get into the interior. Thus, the flannel is wrapped around the beaker so that one edge of the cloth touches the outside of the beveled rim of the beaker. The closed end of the beaker is grasped by means of the other end of the flannel and rotated so that the beaker slides with a rotary motion through the part of the flannel which is wrapped around the beaker below the rim. At the same time this part of the flannel is slowly drawn down toward the bottom of the beaker. Finally, the hand rotating the bottom end of the beaker has to release its grip as the hand with the other end of the flannel moves down. The hand which has become free now grasps the beaker near the opening by means of a piece of chamois, and the other hand finishes wiping the bottom part by means of a rotary motion with the flannel. From now on, touching the beaker with the hands is no longer permitted. By means of chamois the beaker is wiped three times in the described manner from the opening toward the bottom.

The beaker is then placed in the desiccator and allowed to stand for 10 minutes near the open balance case in the closed desiccator. By means of forceps it is then placed on the left-hand pan of the balance. After counterbalancing with the pieces of a set of weights and the rider, the doors of the case are opened, and the experimenter leaves the balance for 3 minutes. The weighing is performed in the usual manner. Wiping and weighing are repeated in the described manner until consecutive weighings agree within the limit  $\pm 3a_s$ .

### EXPERIMENT 63

#### Determination of Water by the Method of Brush and Penfield<sup>17</sup>

$\text{CaSO}_4 \cdot 2\text{H}_2\text{O} : P = 20.90$  per cent water.

$\text{H}_2\text{O} : 100f = 100$  per cent water.

Mass of sample,  $S = 1500a_s^3$

*Required:*

Clear plate of the mineral selenite.

Dry ice or carbon dioxide snow.

Blast lamp.

Centrifuge.

The weighed sample of selenite is ignited in a glass tube, and the water vapor is condensed near the center of the tube. The end of the tube containing the ignited mineral is drawn off, and the remainder of the tube is weighed before and after removal of the condensed water.

A tube *A*, Fig. 68, is made from clean soft-glass tubing of 6-mm outer diameter. The tube is dried in a drying oven for several hours at 110° C and then prepared for weighing by wiping with moist flannel and chamois. Cooling block and cover are removed from the desiccator, Fig. 66, and the tube is placed in the desiccator and thus allowed to stand near the open balance case for 10 minutes. The tube is then placed on the left pan by means of suitable forceps and counterbalanced with weights. Preparation of a permanent tare would not be practicable, since the tube is destroyed in the course of the experiment. The weighing is performed as usual, a microchemical balance being given a 3-minute period of rest between counterbalancing and weighing.

Since cleavage of selenite is easily performed, it is simple to remove a particle of the desired mass from the mineral by means of a penknife. The balance is arrested, and the ignition tube *A* is removed with forceps. The small piece of mineral is introduced into the tube

<sup>17</sup> Anna-Greta Hybbinette and A. A. Benedetti-Pichler, *Mikrochemie* (1941).

and allowed to glide down into the bulb. The thistle tube *f*, Fig. 68, is used only for introducing powders which otherwise would adhere to the walls of the tube *A*.

The tube, which was continuously held with forceps, is immediately returned to the pan of the balance and weighed again. The difference between the two weighings represents the mass of the sample. The tube is removed from the balance and may now be handled with the fingers.

The tube being held at an angle of 45 degrees to the horizontal, a capillary of 0.5- to 1-mm bore is drawn out at *a*, Fig. 68*A*. It is cut off at 5 cm from the taper as shown in Fig. 68*B*. When the tube has

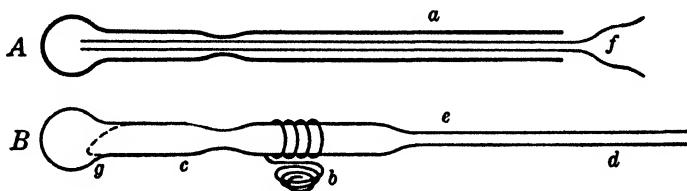


FIG. 68. Determination of water.

cooled to room temperature, a helix *b* is slipped over the tube. The helix is made of 1-mm copper wire which forms the small basket also, Fig. 68*B*. A small piece of dry ice is placed in the wire basket so that it leans against the helical coil. The apparatus is laid aside for 5 minutes, when part *c* of the ignition tube will have become sufficiently cold, and the heating of the sample may be started. First, a blast lamp is adjusted to give a small narrow cone of hot blue flame. The tube is then grasped at the capillary, and the bulb containing the sample is heated slowly over a small Bunsen flame placed close to the blast lamp. The small sample of selenite loses all its water within 2 or 3 minutes. Then by means of a blast lamp, the bulb containing the sample is made to collapse so that the glass encloses the sample. With the aid of a glass rod the collapsed bulb is drawn off so as to seal the tube at *g* as indicated by the broken line in Fig. 68*B*. Not more than 5 minutes is required for the whole process of ignition.

One minute after ignition the dry ice is removed from the wire basket, and the tube is laid aside for approximately 5 minutes to acquire room temperature. Then the tube is sealed by heating the capillary at *d*, Fig. 68*B*, with a small flame, fusing it shut, and drawing off the short end of the capillary. The water, most of which condenses as a large drop at *c*, is now collected in the point of the capillary.

lary by whirling in an electric centrifuge. To prevent breakage, the tube is supported with pieces of cork at the tapered portion near *c*.

The outside of the capillary end is finally cleaned by wiping with moist flannel and chamois. While the capillary is still held with the chamois, it is cut off at *e*, Fig. 68*B*, and weighed immediately. Since the water is located at the sealed end approximately 3 cm from the opening of the capillary, evaporation is too slow to cause a significant loss of weight during weighing. After weighing, the water is removed with the aid of the centrifuge. A plug of clean filter paper is introduced into the point of a clean centrifuge cone. The capillary is taken from the balance with forceps and placed in the centrifuge cone so that the opening rests on the plug of filter paper. Brief whirling in an electric centrifuge removes the water quantitatively from the capillary. The capillary is picked up with forceps and returned to the pan of balance. Since it has not been handled with the fingers, it may be weighed immediately. The weight of the water is obtained as the difference of the last pair of weighings. The weight of the capillary should remain unchanged after drying at 110° C. The result of the determination may deviate for  $\pm 0.5$  per cent water from the calculated water content of selenite.

#### APPARATUS FOR WORKING WITH EMICH'S FILTERSTICK<sup>10</sup>

The two most efficient and most widely used techniques for the handling of precipitates in connection with gravimetric determinations on centigram and milligram samples were developed by Emich and his coworkers. The filterstick,<sup>2, 9, 18</sup> a short narrow tube with a filter mat at one end, is used in conjunction with a microbeaker of approximately 5- to 25-ml capacity. The two are weighed together empty. After the sample has been put in the beaker, another weighing is performed. The sample is dissolved in the beaker, and the weighing form is precipitated. The filter mat of the filterstick is immersed in the mixture of precipitate and solution while the other end of the stick is connected with a siphon operated by suction. The liquid contents of the beaker are removed through the filterstick, and the interior of the beaker and the filterstick are rinsed with a suitable wash liquid which is drawn off through the filterstick. Part of the precipitate is collected on the filter mat, and part remains in the beaker. Filterstick and beaker are dried (ignited), and weighed together. No quantitative transference of the precipitate is required, and only three

<sup>18</sup> H. Häusler, *Z. anal. Chem.*, **64**, 361 (1924).

weighings are necessary, since sample and weighing form are weighed in the same apparatus.

The use of the filter beaker<sup>2, 9, 19</sup> is likewise based on the "principle of three weighings."<sup>20</sup> The filter beaker may be described as a micro teapot. For filtration, suction is applied at the opening of the spout which contains the filter mat close to the pot. The technique of working with the filter beaker is very similar to that described for the filterstick. One might imagine that the latter technique was derived from the former by breaking the spout off the teapot and using it as an immersion filter after inserting it through the top opening of the pot.

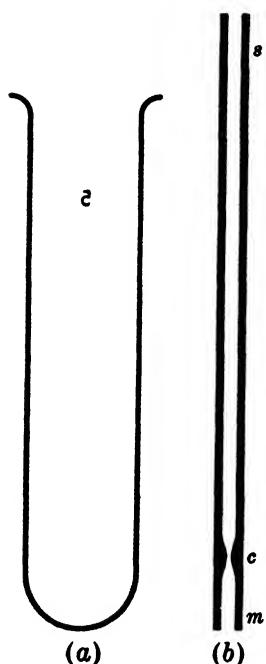


FIG. 69. Microbeaker and filterstick.

the last. In the meantime hours may have lapsed, the equilibrium of the balance may have changed, and the apparatus (the beaker rather than the filterstick) may have been exposed to prolonged attack by corrosive reagents and to the effects of high temperatures. The consequences are obvious, and they are the more noticeable the higher the precision of the balance.

The filterstick, Figs. 69B and 73E, is exposed to the solutions for only a short time, and it is less affected by ignition, since it is inside the beaker during this treatment. Therefore, the filterstick may be made of almost any material which seems otherwise desirable: any

<sup>19</sup> E. Gartner, *Monatsh.*, 41, 477 (1920).

<sup>20</sup> F. Emich and J. Donau, *Monatsh.*, 34, 553 (1913).

kind of glass, quartz, porcelain, platinum, or plastic. The filter mat may be prepared from filter paper (Expt. 69), asbestos,<sup>18</sup> platinum sponge, Alundum, porous porcelain, or fritted glass or quartz.<sup>9</sup> Jolly<sup>21</sup> describes the sealing of Alundum disks into Pyrex tubing. Instructions for sealing unglazed porcelain (Selas, porosity 10) into Pyrex apparatus are given by Holmes.<sup>22</sup> Excellent porcelain filtersticks with mats of porous porcelain may be obtained from the Staatlische Porzellan-Manufaktur in Berlin, Germany.<sup>9</sup> Filtersticks of glass with filter mats of fritted glass are made by Schott and Gen. in Jena, Germany,<sup>9</sup> and by the Corning Glass Works, Corning, N. Y. Filtersticks of platinum provided with mats of platinum sponge may be ordered from any reputable platinum works.<sup>18</sup> The shape of the filterstick is of no importance, provided that the filter mat is securely held in place and that no dead space is created, which impairs the efficiency of washing. The length of the stick must be such that one end is at least 1 cm outside the beaker when the other end touches the bottom of the beaker. This will assure that the rubber tubing used for making the connection with the siphon cannot get in contact with the inside walls of the beaker and remove particles of the precipitate.

The beaker, Fig. 69A, must be made of material which resists chemical attack and the temperatures to which it must be exposed. Chemically resistant glass, which does not tenaciously retain electric charges, is satisfactory, if temperatures higher than 450° C are not required. Crucibles of porcelain or platinum are used when the precipitate must be ignited at higher temperatures. Crucibles serving as microbeakers should not be directly exposed to the flame of a burner or blast lamp, but heated inside a larger crucible. Ignition at temperatures around 1000° C is best performed with the aid of an electric crucible furnace or muffle oven.<sup>18</sup> Even when heated in this manner, the crucibles undergo slight changes of mass. A platinum crucible of 4-gram mass lost 1 γ per minute during ignition at 1000° C in an electric furnace. During the burning of the carbon residue of a charred filter paper this loss was reduced to 1 γ in 5 minutes.<sup>23</sup>

The block shown in Fig. 6 is designed to accommodate two microbeakers and the filtersticks and glass tares belonging to them. The beakers are placed in the holes indicated by the letter *c*. The glass tares are placed in the holes behind the beakers to which they belong. The two holes, indicated by the letters *d*, are designed to hold the

<sup>21</sup> S. E. Jolly, *Ind. Eng. Chem., Anal. Ed.*, **13**, 478 (1941).

<sup>22</sup> F. E. Holmes, *J. Chem. Education*, **18**, 311 (1941).

<sup>23</sup> E. Schwarz v. Bergkampf, *Mikrochemie, Emich-Festschrift*, p. 268 (1930).

filtersticks with the ends containing the filter mats up. Two 3.5-cm pieces of glass tubing of 6-mm outer diameter are sealed at one end and then inserted in the holes *d* with the sealed end down. Thus, the filtersticks cannot come in contact with the wood of the block. The

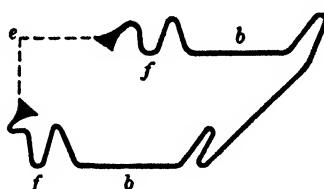


FIG. 70. Aluminum frame for holding microbeaker and filterstick on the pan of the balance.

glass (density 2.5) or from 0.5-mm aluminum wire (density 2.6). The dimensions of the frame depend upon the size of the pan. Its base should be approximately 3 cm square. Figure 70 shows in perspective the frame as it would appear when properly placed upon the

two glass cups lining the holes *d* are removed from the block from time to time and inspected for cleanliness. Previous to weighing, microbeakers are placed in the cavity of the metal block of the desiccator shown in Fig. 66.

A frame, Fig. 70, is used to hold microbeaker and filterstick in place on the pan of the balance.<sup>24</sup> The device is made either from a 1-mm rod of soft

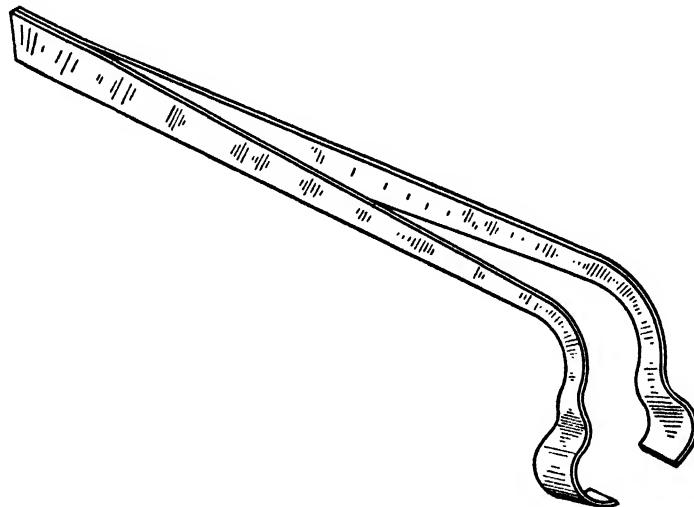


FIG. 71. Tongs for transferring microbeakers.

left-hand pan of the balance. The weight of the frame should not exceed 1 gram. Beaker and filterstick are laid across the parts designated by *b* and *f*, respectively. The ends *e* are flattened so that the frame may be easily picked up with forceps. The frame is carefully

<sup>24</sup> H. K. Alber, *Mikrochemie*, 18, 92 (1935).

cleaned and then placed inside the balance case to be kept there ready for use.

The special forceps, Fig. 71, are shaped to grasp the cylindrical surface of the microbeaker. They are useful in transferring the beaker to the balance. The forceps shown in Fig. 72<sup>24</sup> are designed to give a secure hold on the filterstick. The slightly conical grooves are made to fit around thin cylindrical rods.

The steam bath, Fig. 7, the bath, Fig. 8, and the heating block, Fig. 9, may be used for heating the contents of microbeakers. A clean drying oven may be employed for the drying of beaker and filterstick, if the drying block, Fig. 74, is not available. For the purpose of filtration, the filterstick is connected to a siphon, Fig. 15A, by means of a short piece of clean, soft rubber tubing. Another microbeaker, which is to receive filtrate and washings, is placed inside the suction tube. A trap shown in Fig. 12 should be inserted in the suction line. The three-way stopcock *T* is used for breaking the suction.

For measuring and adding of reagents, measuring pipets of 2-ml capacity divided in 0.1 ml and measuring pipets of 0.2-ml capacity divided in 0.01 ml will serve. They must be cleaned frequently with chromic-sulfuric acid mixture, for which task the suction device shown in Fig. 14B will be found practical. Pairs of handy reagent droppers are obtained from 30-cm lengths of Pyrex tubing (2- to 8-mm bore) by drawing out the middle to a capillary of 0.5- to 1-mm bore. Approximately 10 cm of capillary is retained with each half of the glass tube, and the wide opening as well as the capillary opening of each dropper is carefully fire-polished. The droppers may be crudely calibrated by sucking up water from a 10-ml graduated cylinder. Graduation marks are scratched on the wide tube by means of a file, and the tube is then carefully heated nearly to the softening point to remove the strains produced by the file marks. Such reagent droppers may be continuously kept in a cylinder filled with cleaning solution. Before use, they are carefully rinsed with tap water and distilled water.

The wash bottle shown in Fig. 2 is suited for quantitative work. The measuring of wash liquid is at times facilitated by substituting a 10-ml graduate cylinder for the Erlenmeyer flask of the wash

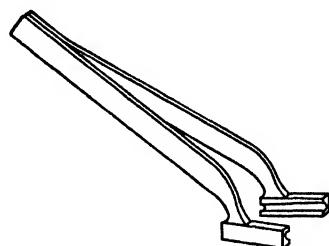


FIG. 72. Forceps for the transfer of filtersticks. H. K. Alber, *Mikrochemie*, 18, 92 (1935).

bottle.<sup>2</sup> Pyrex wash bottles of both types, with glass caps in the place of the rubber stopper, are listed in the Appendix.

### EXPERIMENT 64

#### Preparation of Microbeaker, Filterstick, and Tare

Fiolax glass of Schott und Gen., Jena, is marked with a black stripe in the glass parallel to the axis of the tubing. Microbeakers are best made from Fiolax test tubes of very thin glass. Two beakers approximately 7 cm long as shown in Fig. 69A may be obtained from one test tube of 15-cm length and 16-mm outer diameter. The bottoms are blown up round, and the rims are beveled and carefully fire-polished. Beakers to serve as tare are made in the same way of either Fiolax or soft glass. The bottom of the tare beakers is made flat so that they can be stood on the pan of the balance. After a quick check-up by means of a trip balance to make certain that the tare beakers are not heavier than the microbeakers for which they are intended, the beakers are numbered by etching with hydrofluoric acid so that each tare receives the same number as the microbeaker to which it belongs. Before use, the microbeakers are immersed for 30 minutes in cleaning solution, because complete absence of fat from the interior surface of the beakers greatly facilitates thorough removal of liquids by means of filterstick or siphon. The tare beakers are cleaned, dried, wiped with flannel and chamois, allowed to stand near the balance for 10 minutes, and then transferred into the balance case where they are kept ready for use.

Filtersticks are prepared from soft-glass or Pyrex tubing of 8- to 10-mm outer diameter. Following the directions of Expt. 2, several capillaries of not less than 2-mm outer diameter or more than 12-cm length are drawn out. Short pieces of wide capillary are obtained, which are separated by small bulbs. The capillaries are cut so that each bulb retains on one side 9 cm or more and on the other side at least 5 cm of capillary as indicated in Fig. 73A. The filterstick is formed from the bulb and the longer capillary.<sup>25</sup> The blast lamp is adjusted to give a very narrow, short cone of flame which must not be too hot. While the capillary is continuously rotated, part *c*, Fig. 73A, is heated in the flame. Pulling must be avoided during this operation. It is preferable to push the glass slightly together while it collapses to form the constriction at *c*. A slight bulge at the outside of the tube, resulting from pushing, is easily eliminated by taking the

<sup>25</sup> A. A. Benedetti-Pichler, *Z. anal. Chem.*, **64**, 409 (1924).

glass from the flame while the constriction is still somewhat wider than intended and pulling the tube while the glass is cooling. It is obvious that the capillary must be rotated as long as the glass remains in the plastic state. The tube should assume the shape indicated by Fig. 73B. The narrow flame is now directed on part *d* of the bulb, and the capillary *a* is drawn off so that the bulb becomes rather thin at *e*, Fig. 73C. The bulb is then blown open at *e* as shown by Fig. 73D. The thin bulb *f* is knocked off, and as much of the thin glass as possible is removed from around the opening *o* by lightly touching

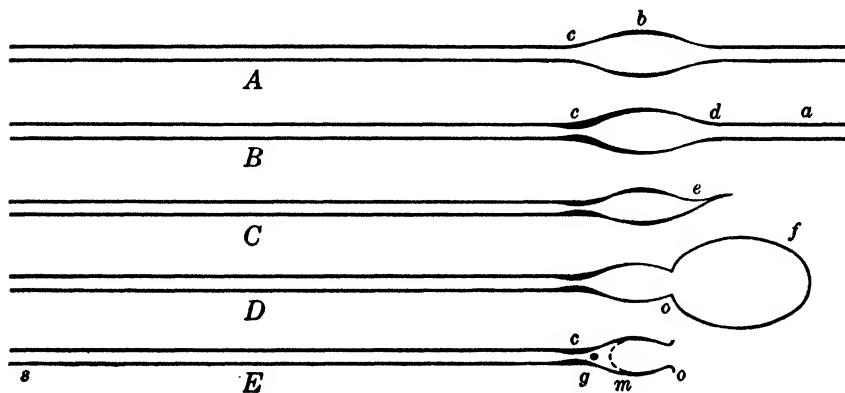


FIG. 73. Preparation of filtersticks, approximately natural size.

with a file. Finally, the filterstick is cut at *s*, Fig. 73E, so that it gets an overall length of 9 cm, and the two openings *s* and *o* are carefully firepolished. If desired, the filtersticks may be etched with hydrofluoric acid so as to show the same numbers as the microbeakers. There is no objection to placing the numbers on the bulbous part. The filtersticks are immersed in cleaning solution for a few hours and then rinsed with tap water and distilled water.

To improve the rate of filtration, the asbestos mat *m*, Fig. 73E, is placed upon an irregular piece *g* of some rigid material which is suitably chemically resistant. This support *g* may be made in many ways. Pieces of fritted glass or porous porcelain or Alundum may be removed from broken crucibles of the Gooch type, funnels of the Büchner type, extraction thimbles, etc. They are thoroughly cleaned with chromic-sulfuric acid, hydrochloric acid, and ammonia. Porcelain and Alundum may be ignited in addition. The pieces are then crushed in a mortar, and irregular particles of approximately 2-mm diameter are collected in a properly labeled screw-cap vial. A rigid and porous support may also be obtained by rolling a platinum wire

of approximately 0.05-mm diameter into a ball of 2- to 3-mm diameter. The ball is ignited before it is introduced into the filterstick. Of course, the platinum support must not be used when solutions which attack platinum are to be filtered, e.g., acid solutions containing chloride and nitrate ions. Finally, for lack of other materials, one may use an irregular bead of glass. A bead of approximately 2-mm diameter is fused at the end of a glass fiber. While still hot, its sides are somewhat flattened by means of platinum-tipped forceps. The bead is finally grasped with the forceps and drawn off the fiber. Care must be taken to place the bead in the filterstick in such a position that it cannot act as a valve and stop the passage of liquid.

The filter mat is prepared from purified asbestos as used in Gooch crucibles. The asbestos must consist of soft, long fibers. Brittle needles, which easily disintegrate to a fine powder, must be absent. The asbestos is used dry. Suitable particles are picked up with forceps and introduced into the filterstick. Not too much should be taken. The mat is finally pressed together by means of a glass rod, moistened with a drop of water, again pressed together, and then connected with the siphon of the suction tube, which contains a large vial for collecting the washings.

The filterstick is now tested by means of a suspension of fine asbestos. A small amount of asbestos is shaken in a microbeaker with distilled water. The mixture is set aside to give the coarse particles time to settle. Finally, the suction flask is placed so that the part of the siphon which carries the filterstick projects beyond the edge of the table top. This position is recommended for all filtrations with the filterstick. Light suction is applied, and the beaker, held in the hand, is raised so that the opening of the filterstick is immersed in the top layer of the asbestos suspension. The suction is increased until a reasonable rate of filtration is obtained, and approximately 0.5 ml of the suspension is allowed to pass through the filterstick. The beaker with the asbestos suspension is removed, the suction is broken, and the filtrate is inspected after removal of the vial from the suction tube. If the filtrate is perfectly clear, the asbestos mat is considered satisfactory.

The filtrate is rejected, and the vial is returned to the suction tube. It may be mentioned that the filterstick need not be taken off the siphon when the suction tube is opened.

The suction is started again, and the outside of the filterstick is rinsed down by means of a stream of water from the wash bottle. Then the microbeaker which is to be used with the filterstick is

grasped with clean fingers and half filled with distilled water. The beaker is moved under the filterstick and raised so that the filterstick touches the bottom of the beaker. If the mat has the proper density, the contents of the beaker will be siphoned off within 1 minute while the suction pump is half turned on. In other words, the rate of filtration should be approximately 5 to 10 ml per minute when a vacuum of 300 mm mercury is applied. As a general rule, mats which give a high rate of filtration are preferable.

When all the water has been removed from the beaker, it is set aside. The top part of the filterstick is grasped with clean fingers, and the filterstick is pulled out of the rubber tubing connecting it to the siphon. Rubber particles adhering to the filterstick are removed with a clean piece of cloth, and then the filterstick is placed in the microbeaker.

The beaker and the filterstick are dried at the temperature used for drying the precipitate in the determination which is to follow. The outside of the beaker is wiped with a clean cloth, and then the beaker, with the filterstick inside, is laid on a clean watch glass and thus transferred into the drying oven.

The drying process may be considerably hastened by means of the drying tube <sup>26</sup> and drying block shown in Fig. 74. The drying block of aluminum is cut in two along the horizontal plane including the axis of the drying tube. The upper half is usually attached to the lower half by means of hinges so that the top can be swung back. The lower half of the block has a thermometer well bored parallel to the cylindrical cavity holding the drying tube. Heating may be done by means of electricity or gas.

Only one part of the drying tube needs explanation. The tube *s*, which is connected to the suction line, has a total length of 35 to 40 cm, i.e., it is 5 to 10 cm longer than the drying tube proper. The tube *s* has an outer diameter of approximately 4 mm; at *n* it is drawn out to an outer diameter of 2 mm, and at *m* a tube of 6-cm length and approximately 6-mm outer diameter, which fits snugly into the stem of the drying tube proper, is joined into *s*. The wide part *m* must be located in the tube *s* so that it produces a tight seal with the rubber tubing at *m* when the microbeaker is all the way in the drying tube as shown in Fig. 74. The drying tube must be cleaned with chromic-sulfuric acid, rinsed with tap water and distilled water, and dried before it is put into use. The rubber stopper with the cotton filter should always be in place to prevent dust from getting into the tube.

<sup>26</sup> A. A. Benedetti-Pichler, *Mikrochemie, Pregl-Festschrift*, p. 6 (1929).

The soft-rubber tubing at *n* must not receive more than a trace of glycerin before it is put in place.

At the start of the working period the drying block is heated to the required temperature, and the heating is then regulated so as to maintain the selected temperature. The drying tube is placed on a sheet of clean paper. Each of the two corks *c* has a straight face at the underside of the tube so that the tube may rest securely on a table top.

When beaker and filterstick are ready for drying, the stopper *f* is removed from the drying tube, and the tube *s* is pushed into the drying tube until the end *n* projects beyond the opening of the wide tube

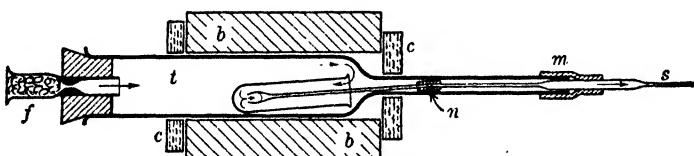


FIG. 74. Drying tube and drying block, one-quarter natural size.

*t*. Without being removed from the beaker, the filterstick is now connected with clean fingers to end *n*. Then the tube *s* is pulled back into the drying tube, care being taken that the microbeaker follows the movement of the filterstick. As soon as the opening of the microbeaker is inside the drying tube, the operation may be finished by holding the drying tube perpendicular while pulling *s* back into the tube. Gravity causes the microbeaker to follow the filterstick into *t*. Finally, the microbeaker and the filterstick should be in the location shown in Fig. 74 while the rubber *n* is outside the heated part of the tube and a tight seal is produced at *m*. The stopper carrying the cotton wad *f* is put in place, and *s* is connected to the suction line. The suction is turned on full, and *then* the drying tube is placed in the drying block *b* as indicated in Fig. 74. The two corks *c* are pressed tightly toward the sides of the block so as to prevent rolling of the tube *t*.

The drying is considerably speeded up by the current of air which enters through *f* and continues on its way through the microbeaker and the filterstick as shown by the arrows in the figure.

To remove the microbeaker from the drying tube, the tube is taken from the drying block without interrupting the suction. It is again placed on a sheet of clean paper. Stopper *f* is removed, and the microbeaker is transferred to the paper by pushing tube *s* into the tube *t*. The filterstick, without being removed from the beaker, is discon-

nected from *n*. Now the suction is broken, tube *s* is pulled back to obtain the tight seal at *m*, Fig. 74, and the stopper *f* is put into place. The drying tube remains outside the heated block.

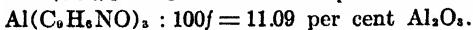
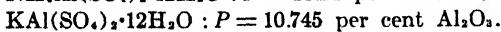
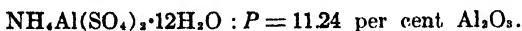
The end of the filterstick which was connected to *n* is carefully cleaned with a cloth. Without removing the filterstick from the beaker, the outside of the beaker is wiped with moist flannel and chamois as outlined in Expt. 62. The microbeaker with the filterstick inside is then placed in the desiccator, Fig. 66, which is closed and placed near the open case of the balance. After 10 minutes the beaker is transferred to the pan of the balance by means of forceps. Using the frame, Fig. 70, the beaker is placed across the bars *b*. The filterstick may remain in the beaker.

For the preparation of the tare, the tare beaker is transferred to the right-hand pan. Glass rods of approximately 4-mm and 1-mm diameter are held ready. They should consist of soft glass, and they are cleaned with flannel and chamois as customary and then placed on a sheet of clean paper. Holding the rods by means of a piece of chamois and breaking it by means of pliers, one cuts off pieces of suitable lengths. The rider remains at zero. First a rather long piece of heavy rod is placed on the right-hand pan next to the tare beaker. A quick trial shows whether the tare is too heavy or too light. If it is too light, the piece of rod is transferred into the tare beaker and a new piece of rod is placed on the pan. If the piece of rod adds too much to the mass of the tare, it is removed from the pan and a shorter piece of rod is tried. The adjustment is finished with the rod of 1-mm diameter and finally with glass thread. The tare is adjusted so that equilibrium is attained when the rider is located between 0.5 and 1 mg.

Finally, the doors of the balance are opened, and the balance is given a rest period of 10 minutes before the weighing is performed. The constancy of the mass of filterstick and beaker may be proved by filling the beaker with distilled water, drawing it off through the filterstick, and repeating the drying and weighing procedures.

## EXPERIMENT 65

### Determination of Aluminum Oxide in Alum<sup>5, 26</sup>



$$\text{Log } 100f = 1.04510$$

$$\text{Mass of sample, } S = 400a_s^3$$

Weighing form dried at 140° C.

*Required:*

Alum,  $\text{NH}_4\text{Al}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  or  $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ . Microscopic inspection of the salt is advisable. If white, opaque spots indicate partial dehydration, a small quantity of the salt is recrystallized; see Appendix.

Hydrochloric acid, concentrated, pure, clear; see Appendix.

Ammonium acetate, 2 M clear.

Oxin reagent, clear; see Appendix.

The alum is dissolved in dilute hydrochloric acid and treated with a sufficient excess of 8-hydroxyquinoline. Slow addition of ammonium acetate at 100° raises the pH so as to produce a quantitative precipitation of the aluminum oxyquinolate. The precipitate is freed from the mother liquor, washed, dried at 140°, and weighed. The description of the procedure follows.

**WEIGHING OF BEAKER AND SAMPLE.** Filterstick and beaker are cleaned, dried, and made ready for weighing as described in the preceding experiment. The beaker is placed across the bars *b*, Fig. 70, of the frame which has been previously put on the pan of the balance. The filterstick is then taken out of the beaker by means of suitable forceps and laid across the hooks *f* of the frame. Using the prepared tare bottle for counterbalancing, beaker and filterstick are weighed. After the balance has been arrested the front window of the case is opened, and, by means of a microspatula or a penknife, the desired amount of alum is introduced into the beaker. For work with a microchemical balance, 2 to 5 mg should be taken; with analytical balances the approximate amount of sample is calculated from the equation  $S = 400a_s$ . The microbeaker need not be removed from the pan of the balance if it has been placed in such a way that the opening of the beaker projects over the edge of the pan. If particles of the sample are lost during transference, they will drop on the base of the balance case and cause no error in the weighing to follow.

By means of tare, rider, and, if necessary, calibrated 10-mg and 20-mg pieces, the weight of sample plus beaker, filterstick, and frame is determined. The balance is arrested, and, after the front window is opened, beaker and filterstick are picked up with clean fingers without touching either the frame or the pan. The beaker is picked up so as to bring it immediately into an upright position. Lightly knocking the outside of the beaker with the spatula causes particles of the sample to drop to the bottom of the beaker, which is then placed in a wooden block. The filterstick is grasped so that it is immediately swung into the upside-down position, i.e., the perpendicular position with that end up which contains the filter mat. Thus the accidental loss of asbestos fibers is prevented. The filterstick is placed upside down in the wooden block and kept there under cover until needed.

**SOLUTION OF SAMPLE AND PRECIPITATION OF THE WEIGHING FORM.**

The alum is dissolved in 0.2 *M* hydrochloric acid, and 0.15 ml of it is taken for each milligram of alum. The acid is added dropwise by means of a measuring pipet. While the inclined microbeaker is slowly rotated between the fingers, the drops are made to land just below the rim of the beaker. In flowing down, the drops rinse the walls of the beaker. To hasten solution of the sample, the beaker is briefly heated in the steam bath, and its contents are mixed by rotating the beaker. Then, 0.03 ml of oxine reagent is added for each milligram of alum present. The beaker is placed on the steam bath, and 2 *M* ammonium acetate is added dropwise from a pipet, mixing after each addition, until a permanent turbidity is formed. The mixture is then heated on the steam bath until the precipitate appears distinctly crystalline, which requires approximately 1 minute. The dropwise addition of ammonium acetate is continued until the precipitation appears complete. As a rule, 1 or 2 drops of the reagent suffice to reach this state. Finally an excess of the 2 *M* ammonium acetate is added, and exactly 0.08 ml of it is to be used for each milligram of alum. The contents of the microbeaker are heated for 10 minutes on the steam bath, after which the hot solution is drawn off through the filterstick.

**FILTRATION AND WASHING.** The period of digestion is used for connecting the filterstick to the siphon. The filterstick is picked up with clean hands and held in the upside-down position while a drop of water from the wash bottle is placed on the opening above the filter mat. The drop may either seal the whole cross section of the opening, or it may flow down to wet the filter mat. In either event, loss of asbestos is made impossible when the filterstick is inverted and connected to the siphon. The suction tube, which contains a large vial for collecting filtrate and washings, is moved forward so that the microbeaker will easily clear the edge of the table top. The microbeaker is grasped with the fingers and raised up so that the filterstick dips into the solution above the precipitate. No more suction is applied than just necessary to give reasonably rapid filtration. If the rate of filtration drops after some precipitate has collected in the filterstick, it may be increased by applying stronger suction. When most of the clear solution has been drawn off, the beaker is raised so that the filterstick touches the bottom of the microbeaker, and the liquid is sucked off as completely as possible. If the walls of the microbeaker are not sufficiently clean, some drops will remain on the upper parts while the bottom part of the beaker is reasonably dry. This can be remedied by moving the beaker so that the head of the

filterstick wipes these drops together and they flow to the bottom where they may be easily taken up by the filterstick. The same procedure should be followed when washing the beaker.

Hot water is used for washing—not more than 0.25 ml per mg of alum taken. This total amount of wash liquid is applied by means of a wash bottle in four equal portions, each time proceeding as follows: While the head of the filterstick still touches the bottom of the microbeaker, the whole length of the filterstick is rinsed down with 3 drops of water, which are immediately sucked into the filterstick when they reach the bottom of the beaker. Then without breaking the suction, the beaker is removed from the filterstick and rotated in an inclined position while the remainder of the portion of wash liquid is added dropwise. The nozzle of the wash bottle must never touch the walls of the microbeaker, and the drops are supplied in such a manner that they land close below the rim of the beaker. If one does not succeed in rinsing the whole inside of the beaker in this manner, then it must be accomplished after adding the whole of the portion of wash water. The beaker is first briefly heated by holding it in the steam escaping from the bath. Then it is inclined and rotated so that the wash water flows across each part of the inside walls. Finally the beaker is placed for 1 minute on the steam bath, and then its liquid contents are removed as completely as possible through the filterstick. As already stated, this routine is followed four times.

**DRYING AND WEIGHING THE PRECIPITATE.** From now on the filterstick must remain continuously in the microbeaker. Before it is removed from the siphon, the suction is gradually broken by means of the stopcock *T* of the trap, Fig. 12. Then one hand grasps the beaker between the last three fingers and the palm. The top end of the filterstick is held with thumb and forefinger of the same hand. The short rubber tubing on the end of the siphon is held in place with the other hand, and the filterstick is pulled out of the tubing. Microbeaker and filterstick are then transferred into the drying tube and heated for 5 minutes at 140° C while air is being sucked through the tube. Drying, wiping, and weighing follow the technique outlined in Expt. 64. If a drying oven is used, the drying period must be extended to 10 or 15 minutes.

**TESTING THE FILTRATE.** The filtrate which has been collected in the vial inside the suction tube is inspected for particles of the precipitate and asbestos fibers.

**CLEANING OF BEAKER AND FILTERSTICK.** To prepare the apparatus for another determination, the microbeaker is filled with 6 *M* hydrochloric acid. After heating on the steam bath, the hot acid is drawn

off through the filterstick. Beaker and stick are finally rinsed with hot water.

The result of the determination should agree with the theoretical content on alumina within the limits  $\pm 0.12$  per cent  $\text{Al}_2\text{O}_3$ .

## EXPERIMENT 66

### Determination of Calcium Oxide in Iceland Spar<sup>5</sup>

$\text{CaCO}_3$ , Iceland spar :  $P = 56.03$  per cent  $\text{CaO}$ .

$\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O} : 100f = 38.38$  per cent  $\text{CaO}$ .

$\text{Log } 100f = 1.58410$ .

Mass of sample,  $S = 400a_r$ .<sup>3</sup>

Weighing form dried between  $100^\circ$  and  $105^\circ$  C.

*Required:*

12 M hydrochloric acid.

3 per cent solution of oxalic acid.

2 M ammonia.

The details of manipulation need not be repeated; they were described in the preceding experiment. The Iceland spar is used in powder form. From 3 to 5 mg of it should be taken when working with a microchemical balance.

**SOLUTION OF SAMPLE.** The sample in the microbeaker is first covered with 1 ml of distilled water. Then, with the beaker inclined at an angle of approximately 30 degrees to the horizontal, 12 M hydrochloric acid is added drop by drop until the sample is completely dissolved. The drops are made to land below the rim of the beaker so that the acid flows down slowly along the wall. The spray caused by the evolution of carbon dioxide is caught on the walls of the beaker if the beaker is held properly inclined. Formation of a stable, fine spray by the liberation of gases from solutions is a source of serious losses if escape of the spray from the apparatus is not prevented. In addition to the inclination of the beaker, other precautions are required to counteract the undesirable effects of the phenomenon: the sample is covered with a relatively large volume of water, the acid is added slowly, and it is added in a manner which will assist the formation of a layer of relatively concentrated solution close to the bottom of the beaker. Of course, everything should be avoided which tends to render the contents of the microbeaker homogeneous.

**PRECIPITATION OF CALCIUM OXALATE.** When solution is complete, the dissolved carbon dioxide is removed by heating. Without mixing the contents, the inclined microbeaker is exposed to the steam escaping from the steam bath. When the liberation of carbon dioxide has

come to an end, the contents of the microbeaker are mixed by swirling. Then such a volume of 3 per cent oxalic acid is added that 0.15 ml of it is used per milligram of sample taken. The solution is added dropwise near the rim of the beaker, which is slowly rotated. The use of reagents for rinsing apparatus facilitates keeping volumes within the desired limits.

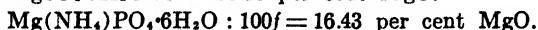
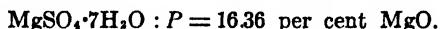
After a very small drop of methyl red solution has been added by means of a capillary pipet, the solution in the microbeaker is treated with 2 *M* ammonia, added dropwise, with mixing after each addition, until a permanent turbidity is obtained. The mixture is then heated on the steam bath until the turbidity dissolves, and then the dropwise addition of 2 *M* ammonia is continued on the steam bath until it seems that one or two drops more would produce a permanent turbidity in the hot solution. Then, 1 *M* ammonia is added dropwise to the solution on the steam bath, with mixing after each addition, until the color of the solution changes to yellow. The contents of the beaker are thoroughly mixed by swirling. The beaker is left for 5 more minutes on the steam bath. It is then transferred to the wooden block and allowed to stand under cover for 1 hour.

**DECANTATION THROUGH THE FILTERSTICK.** The clear solution is then decanted through the filterstick so that as little of it as possible is left with the precipitate in the beaker. In a similar manner, the precipitate is washed with four 0.75-ml portions of cold water. Only after the last washing is the main portion of the precipitate allowed to enter the filterstick. Washing by decantation is always practiced when the precipitate has the tendency to clog the filter mat. Drying by means of the drying tube requires 10 minutes at 100° to 105° C.

The result should not deviate more than  $\pm 0.6$  per cent CaO from the expected value. Beaker and filterstick are prepared for further use by washing with hot 6 *M* hydrochloric acid as outlined in Expt. 65.

## EXPERIMENT 67

### Determination of Magnesium Oxide in Magnesium Sulfate<sup>5, 27</sup>



$$\text{Log } 100f = 1.21564.$$

$$\text{Mass of sample, } S = 400a,^3$$

Weighing form dried at room temperature after rinsing with alcohol.

<sup>27</sup> A. A. Benedetti-Pichler and F. Schneider, *Mikrochemie, Emich-Festschrift*, p. 1 (1930).

**Required:**

Drying tower filled with calcium chloride hexahydrate.

Magnesium sulfate heptahydrate. The salt must be inspected under the microscope. If there are signs of dehydration, it is recrystallized from water. See Appendix.

**6 M** ammonia.

Ammonium chloride, 30 grams per liter solution.

Sodium phosphate, 100 grams  $\text{Na}_3\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  per liter solution.

Methyl alcohol or ethyl alcohol, 95 per cent or stronger. A few milliliters of the alcohol must leave no visible residue when evaporated on a watch glass.

A sample of 2 to 5 mg of the magnesium sulfate heptahydrate is taken if a microchemical balance is used. The salt is dissolved in the microbeaker by adding 0.2 ml of ammonium chloride solution for each milligram of sample. The beaker is placed for 3 minutes on the steam bath. In the meantime, a small amount of a mixture of equal volumes of 6 *M* ammonia and sodium phosphate solution is prepared. For each milligram of sample used, 0.04 ml of this mixture is taken up with a pipet. The beaker is removed from the steam bath, and the calculated volume of the mixture is run into the beaker. The contents of the beaker are mixed well. The microbeaker is placed in the wooden block and allowed to stand under cover for 6 hours. At the end of this time the originally gelatinous precipitate must be completely converted into coarsely crystalline magnesium-ammonium phosphate hexahydrate. Frequently, this precipitate consists of needles 1 to 3 mm long. If the precipitate should remain partly or completely gelatinous, titration of the ammonia reagent will usually show that its concentration is higher than 6 *M*. Of course, there is no purpose in finishing the determination if the precipitate fails to crystallize.

The liquid is removed completely from the crystalline precipitate. The latter is washed with four 1-ml portions of 0.6 *M* ammonia. Each time the wash liquid is completely removed by means of the filterstick. Finally, filterstick and microbeaker are rinsed with a 1-ml portion of alcohol. The suction is broken while there is still a small amount of alcohol left in the microbeaker, and beaker and filterstick are transferred into the drying tube. The tube *f*, Fig. 74, which contains the wad of cotton is connected to the top of the drying tower by means of a rubber tubing, and suction is started. The air enters at the bottom of the drying tower, passes through the tower, and finally through the drying tube, microbeaker, and filterstick. The drying tube is *not* heated. After 10 minutes, beaker and filterstick are removed from the drying tube and prepared for weighing as usual.

The deviation from the expected value should not exceed  $\pm 0.25$  per cent MgO. Filterstick and beaker are made ready for further use by means of hydrochloric acid and water.

### EXPERIMENT 68

#### Determination of Calcium Oxide and Magnesium Oxide in Limestone<sup>28</sup>

Limestone: 30 to 40 per cent CaO.

2 to 20 per cent MgO.

$\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O} : 100f = 38.38$  per cent CaO.

$\text{Mg}(\text{NH}_4)\text{PO}_4 \cdot 6\text{H}_2\text{O} : 100f = 16.43$  per cent MgO.

Mass of sample: To arrive at an exact figure, it should be calculated separately for the determination of calcium and for the determination of magnesium.<sup>3</sup> The higher figure should be taken. If the limestone contains between 30 and 40 per cent calcium oxide and from 4 to 20 per cent magnesium oxide, the mass of sample may be calculated from the approximate equation  $S = 400 \frac{16}{P} a_s$ , in which  $P$  is the percentage of magnesium oxide in the limestone.

*Required:*

Platinum crucible of 10- to 15-ml. capacity.

Pyrex bulb and steam bath shown in Fig. 75.

2 microbeakers with filtersticks, and 3 more filtersticks.

Microbeaker of clear fused quartz of 15-ml capacity.

Gas washing bottle containing 50 ml concentrated ammonia to which a few pellets of sodium hydroxide have been added.

Limestone, finely ground and carefully mixed.

Mixture, 1 : 1, of sodium carbonate and potassium carbonate, both anhydrous and of tested purity.

Sulfuric acid, concentrated, pure.

Bromine water.

Ammonium citrate, solid, c.p.

Reagents used in Expts. 66 and 67.

Pure air at low pressure.

The limestone is fused briefly with a mixture of sodium carbonate and potassium carbonate. The fusion is dissolved in hydrochloric acid, and the solution is evaporated to dryness. The silica is made insoluble by baking in a drying oven, and then the residue is dissolved in dilute acid. The insoluble silica is eliminated by drawing the solution through a filterstick. The filtrate is oxidized with bromine and then made slightly alkaline by blowing air which contains some ammonia gas on the surface of the heated solution. The precipitate of hydrated oxides of iron and aluminum, which contains the rest of the silica, is removed by siphoning through a filterstick into the microbeaker serving for the determination of calcium. The calcium is weighed as the oxalate monohydrate.

<sup>28</sup> A. A. Benedetti-Pichler, A. J. Llacer, and J. A. Sozzi, *Anales farm. bioquím.* (Buenos Aires), 12, 13 (1941).

The filtrate from the calcium determination is collected in the quartz tube and evaporated to dryness. The ammonium salts and the oxalic acid are removed from the residue by ignition and heating with concentrated sulfuric acid. The residue is finally dissolved. The solution is filtered by siphoning through a filterstick into the beaker intended for the determination of magnesia. Some ammonium citrate is added to keep in solution any iron which may have been accidentally introduced, and then magnesium ammonium phosphate is precipitated.

Microbeakers and filtersticks for the determination of calcium and magnesium are cleaned, but not dried, and held ready in the wooden block. Three more filtersticks, carefully prepared and cleaned, are held ready in stoppered test tubes, which should be labeled to indicate the use for which the sticks are intended.

The platinum crucible is cleaned, briefly ignited over a non-luminous Bunsen flame, and, after cooling to approximately 100° C, transferred to the cooling block of the desiccator. After standing for 15 minutes near the open balance case, the crucible is weighed. It is then placed on a sheet of clean paper, and the calculated amount of limestone is placed on the bottom of the crucible. From 3 to 5 mg of limestone should be taken when a microchemical balance is used. The crucible with the limestone is weighed again and then returned to the paper. By means of a spatula or penknife, a volume of the sodium carbonate-potassium carbonate mixture equal to 1.5 times the volume of the sample of limestone is placed on top of the limestone. It is desirable to have the contents of the crucible collected in a small pile. If it happens that the substances get spread out over the bottom of the crucible, they may be collected by holding the crucible somewhat inclined and tapping it lightly.

**FUSION.** The platinum crucible is picked up with the fingers and placed on a clean triangle supported by a ring stand. The Pyrex bulb *B*, Fig. 75, is placed on the opening of the crucible so that the projection *b* does not touch the wall of the crucible. That part of the bottom of the crucible which holds the limestone-carbonate mixture is heated with the point of a non-luminous Bunsen flame approximately 1 cm high. The fusion is finished within 1 minute, after which time the flame is removed and the crucible is allowed to cool to room temperature.

**SEPARATION OF SILICA.** The bulb is grasped at the handle *a*, Fig. 75, and lifted a few millimeters so that the projection *b* touches the wall of the crucible. The bulb is then rinsed into the beaker with 0.1 ml of distilled water. A tiny drop of indicator solution (methyl red) is added to the bulb by means of a capillary pipet and rinsed

into the crucible with 6 *M* hydrochloric acid, which is deposited drop-wise near the equator of the bulb so that the bulb is rinsed off into the crucible. The hydrochloric acid is added slowly while the bulb is held close to the opening of the crucible, and the adding of acid is stopped when the solution in the crucible turns red. Approximately 0.25 ml of the acid will be required.

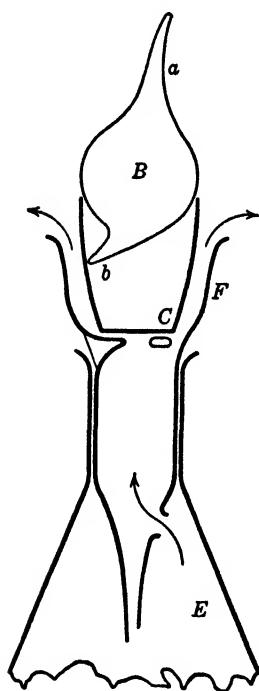


FIG. 75. Steam bath for 15-ml platinum crucibles.

The bulb is placed over the opening of the crucible so that the projection *b* touches the wall. The crucible is transferred to the steam bath shown in Fig. 75, which is very similar to the one illustrated by Fig. 7. The crucible is heated for 5 minutes. By this time the evolution of carbon dioxide will have come to an end. The bulb is lifted a few millimeters so that the projection touches the wall of the crucible, and it is rinsed with 6 drops of distilled water from the wash bottle, which are deposited around the equator of the bulb. After rinsing, the bulb is laid aside, and a stream of clean air is blown on the surface of the liquid in the crucible by means of a capillary, Fig. 28. Heating on the steam bath is continued until the contents have evaporated to dryness. The crucible is again covered with the bulb, which has been wiped dry with a clean cloth, and it is then placed for 40 minutes in a drying oven heated to a temperature between 115° and 120° C. The time is used for drying and weighing the microbeaker and filterstick which are to serve for the determination of calcium.

The crucible is taken from the drying oven, and its contents are treated with 0.4 ml of 6 *M* hydrochloric acid. As usual, the acid is used to rinse the surface of the bulb into the crucible, which is finally covered with the bulb and placed on the steam bath. The contents of the crucible are heated for 15 minutes. If partial evaporation takes place, 6 *M* hydrochloric acid is added from time to time so as to keep the volume constant. Finally, 0.4 ml of distilled water is added, and the heating is continued for 5 more minutes. In the meantime, the microbeaker of clear fused quartz is placed in the suction tube, and the filterstick for the separation of silica is attached to the siphon. After the bulb is removed from the platinum crucible, the crucible is

taken from the water bath and the transfer of the liquid from the crucible to the quartz beaker is started with slight suction. Crucible and filterstick are rinsed with five 0.15-ml portions of hot 0.1 *M* hydrochloric acid. The rinsings are collected with the filtrate in the quartz beaker.

**OXIDATION WITH BROMINE.** All iron is converted to the ferric state by oxidation with bromine before precipitation with ammonia. The bottle containing bromine water is slightly inclined so that the bromine *vapor* flows from the opening into the quartz beaker. The contents of the beaker are mixed by swirling from time to time. When color and odor indicate that an excess of bromine has been added, the quartz beaker is placed on the steam bath and a stream of air is blown on the surface of the solution in the beaker until the excess of bromine has been expelled. Hardly more than 1 minute will be required.

**PRECIPITATION WITH AMMONIA, ELIMINATION OF IRON, ALUMINUM, AND THE REMAINDER OF THE SILICA.** A small drop of methyl red solution is added to the solution in the quartz beaker. If the red coloration fades immediately, the treatment for the elimination of bromine must be continued for another minute, and then another droplet of indicator solution is added. This is continued until the solution stays red. Then the gas wash bottle containing ammonia and a few pellets of sodium hydroxide is inserted in the air line so that the air bubbles through the ammonia and air laden with ammonia leaves the outlet tube. The air-ammonia mixture is blown onto the surface of the solution in the quartz beaker, which is heated on the steam bath. A separate capillary, Fig. 28, should be reserved for this purpose. The treatment is stopped when the solution in the quartz beaker turns yellow. The beaker is immediately covered by inverting a wide vial over its opening, and its contents are heated for 2 more minutes on the steam bath. If the color of the indicator should change to brown or red, some more ammonia must be blown into the beaker.

In the meantime, the microbeaker for the determination of calcium is placed in the suction tube, and the filterstick for the elimination of the oxide mixture is attached to the siphon. The solution in the quartz beaker is transferred to the microbeaker by means of slight suction. The quartz beaker and the filterstick are rinsed with six 0.1-ml portions of a solution of 30 grams of ammonium chloride in 1 liter of water and, finally, with one 0.1-ml portion of distilled water. Each time, the beaker is placed for 30 seconds on the steam bath before the wash liquid is sucked off.

**DETERMINATION OF CALCIUM OXIDE.** The microbeaker containing filtrate and washings is taken from the suction tube after breaking the suction slowly. Its contents are treated with 0.35 ml 6 *M* hydrochloric acid and with 0.60 ml 3 per cent oxalic acid *for each 5 mg of limestone taken*. The solution is then treated by the dropwise addition of 6 *M* ammonia, mixing from time to time, until a permanent turbidity is obtained. Then the beaker is placed on the steam bath, and its contents are heated, being swirled from time to time, until the turbidity has dissolved. While the beaker remains on the steam bath, its contents are diluted with distilled water to a volume of approximately 6 ml. Then, 0.6 *M* ammonia is added dropwise to the hot solution, with mixing after each addition, until the color of the solution in the microbeaker changes to yellow. The beaker is placed on the wooden block and allowed to stand under cover for 1 hour. This time is used to rinse with alcohol the microbeaker and filterstick intended for the determination of magnesia, and to dry and weigh them. The microbeaker of clear fused quartz is carefully cleaned and then placed in the suction tube. It is to receive the filtrate from the calcium oxalate precipitate.

The calcium oxalate is filtered as outlined in Expt. 66. Beaker, filterstick, and precipitate are washed with five 0.5-ml portions of cold distilled water. Drying and weighing are performed as directed in Expt. 66.

**ELIMINATION OF AMMONIUM SALTS AND OF OXALIC ACID.** The quartz beaker containing filtrate and washings is placed on the steam bath, and its contents are acidified by adding 1 drop of 6 *M* hydrochloric acid and then evaporated to dryness. Evaporation is speeded up by blowing a stream of air on the surface of the heated liquid.

When the residue has become perfectly dry, the quartz beaker is removed from the steam bath and placed in a test tube clamp made of wire. The beaker is held inclined at an angle of 20 degrees to the horizontal and fanned with the just non-luminous flame of a Bunsen burner until the ammonium salts are removed. The temperature should not be raised above approximately 400° C. Oxalic acid sublimes from one part of the quartz beaker to the other and is very difficult to remove by heating only. Thus, the beaker is allowed to cool to room temperature, and then approximately 0.05 ml of concentrated sulfuric acid is introduced. By means of a small flame the acid is made to distil up the walls of the beaker, and by proper heating and inclining of the beaker the acid is made to flow over all sublimes of oxalic acid. Finally, the whole tube is heated by fanning with the

flame until all the sulfuric acid is removed. The last operation may be hastened by blowing a light stream of air into the beaker.

After the quartz beaker has cooled to room temperature, its contents are dissolved by adding 0.2 ml of 3 *M* hydrochloric acid for each milligram of limestone taken for analysis. The acid is added drop-wise just below the rim of the inclined beaker, which is rotated so that the successive drops of solvent rinse the whole walls of the beaker. The beaker is placed on the steam bath for 1 minute. It is then picked up with the fingers and inclined and rotated so as to rinse the whole interior with the hot acid. Immediately afterwards, it is briefly centrifuged to collect the contents quantitatively at the bottom of the quartz beaker.

The microbeaker intended for the determination of magnesia is now placed in the suction tube, and the filterstick intended for the removal of turbidity is connected to the siphon. The head of the filterstick is cautiously brought to the bottom of the quartz beaker, and the solution is completely siphoned into the microbeaker. Quartz beaker and filterstick are rinsed with five 0.2-ml portions of distilled water. Each portion of wash liquid is first made to wet the whole interior of the quartz beaker, and it is then collected at the bottom by means of the centrifuge and sucked off through the filterstick.

Other procedures which permit thorough washing of a large surface with small volumes of liquid may be employed. The wash liquid may be sprayed on the walls of the beaker by means of an atomizer, or each portion of wash liquid may be heated to boiling so that the reflux rinses the walls.

The microbeaker is removed from the suction tube, and its contents are carefully mixed by swirling. After the addition of a small drop of methyl red and approximately 1 mg of solid ammonium citrate, the microbeaker is placed on the steam bath, and its contents are neutralized by blowing air laden with ammonia into the beaker until the color of the solution changes to yellow. For each milligram of limestone taken, 0.04 ml of the 1 : 1 mixture of 6 *M* ammonia and sodium phosphate solution is now added, and the determination of magnesium is concluded exactly as directed in Expt. 67.

The results for calcium oxide should agree within  $\pm 0.5$  per cent CaO and those for magnesia within  $\pm 0.3$  per cent MgO with the results of reliable macroanalyses.

## EXPERIMENT 69

Determination of Silica in Limestone<sup>23, 29</sup>

Limestone : 2 to 5 per cent  $\text{SiO}_2$ .

$\text{SiO}_2$  :  $100f = 100$  per cent  $\text{SiO}_2$ .

Mass of sample,  $S = \frac{10,000}{P} a_s$ , with  $P$  for the percentage of  $\text{SiO}_2$  in the limestone, limits the relative maximum deviation to  $\pm 60$  parts per thousand.<sup>3</sup>

Weighing form ignited 10 minutes at  $850^\circ \text{C}$ .

*Required:*

Microchemical balance or analytical balance of very good precision,  $a_s < \pm 20 \gamma$ .

Platinum crucible of 10- to 15-ml capacity with cover.

Forceps with platinum tips.

Glass bulb; quartz triangle; suitable steam bath, Fig. 75.

Electric crucible furnace or blast lamp.

Aluminum heating block or sheet of nickel 10 cm by 15 cm.

Limestone, finely ground and carefully mixed, containing 2 to 5 per cent of silica.

Sodium carbonate, anhydrous, tested purity.

6 M hydrochloric acid.

Sulfuric acid, concentrated, no residue.

Hydrofluoric acid, no residue.

Filter paper for quantitative work, fast grade. No. 589<sup>1</sup> Black Ribbon, of C. Schleicher and Schüll, is recommended.

The limestone is fused with sodium carbonate, and the fusion is dissolved in hydrochloric acid. Silica is made insoluble by evaporation and baking of the residue. The extract of the residue is removed through a paper filter which is added to the silicic acid in the crucible. After ashing the paper, the contents of the crucible are treated with sulfuric acid and then ignited. The crucible with the raw silica is weighed. The silica is volatilized by fuming with sulfuric and hydrofluoric acids, and the loss of weight is used as a measure of the silica present in the limestone.

**PREPARATION OF THE FILTERSTICK.** The usual technique of working with the filterstick presents disadvantages whenever the collected weighing form must be subjected to fusion or a treatment with hydrofluoric acid. The technique developed by Schwarz v. Bergkampf,<sup>23, 30</sup> which is employed in this experiment, meets the special requirements.

<sup>23</sup> Anna-Greta Hybbinette has applied the technique to the microdetermination of silica and mixed oxides in limestone. An analytical balance was used.

<sup>30</sup> Variations of the idea of Schwarz v. Bergkampf have been described by Earl J. King, *Analyst*, 58, 325 (1933), and by Christina C. Miller, *J. Chem. Soc.*, 1939, 1962. The latter uses for a filter mat a disk of 3.5-mm diameter and 1.5-mm thickness cut from Whatman ashless filtration accelerator. Precipitates which must be dissolved for further treatment offer no difficulties. If, however,

The filterstick is made from a piece of soft-glass tubing of 2-mm bore, 4-mm outside diameter, and approximately 20-cm length. The blast lamp is adjusted to give a short, narrow cone of rather cool flame. The glass tubing is made to collapse at the middle so that a short capillary constriction of 0.5- to 1-mm bore is formed. When the tube has cooled to room temperature, it is evenly cut on one side at a distance 8 to 10 mm from the center of the constriction and on the other side approximately 7 cm from the constriction. The resulting tube is shown in Fig. 69B. It is important that the cylindrical form of the original tube be retained for approximately 5 mm at the head *m* of the filterstick. The cut surfaces at *m* and *s* are carefully firepolished by touching the glass for short periods to a hot flame. Special care must be taken that the opening at *m* does not collapse at all, but retains the bore of the original tubing.

The cutting surfaces of a pair of scissors are carefully wiped clean with filter paper, and the hands are thoroughly washed before picking up a clean disk of quantitative filter paper and cutting it into strips 6 mm wide and approximately 5 cm long. One strip of filter paper is rolled between the fingers to a tight little cylinder which probably is too thick to fit into the head *m* of the filterstick. The proper length of the strip of paper is determined by partly unrolling the little cylinder and cutting off short pieces of paper until the cylinder fits the tube tightly. The cylinder is opened again, and the required length of the strip is measured. Several strips are cut to the required length, and several pieces are cut to a length of 12 mm. Two of these 12-mm pieces serve for the wiping of the filterstick, and the total weight of one strip and two rectangular pieces 6 mm by 12 mm is determined on an analytical balance. After weighing a whole disk of the filter paper, the ash content of the fraction of the disk used in a micro-determination is calculated.

Finally, the hands are again carefully washed previous to rolling a cylinder from a fresh strip of paper. The cylinder is introduced into the head *m* of the filterstick. It must fit *tightly*, and when pushed all the way in, a 1-mm length of the cylinder should project beyond the opening at *m* so that the paper cylinder can be easily grasped for removal after filtration. The filterstick is best kept in a clean, stoppered test tube until it is needed.

a gas is liberated during the solution of the precipitate, it may happen that the asbestos mat in the filterstick is disintegrated. The difficulty is easily overcome by using fritted glass or porous porcelain in place of the asbestos mat. Finally, a filterstick with two asbestos mats may be used. A. A. Benedetti-Pichler, *Z. anal. Chem.*, **70**, 293 (1927).

**FUSION.** Platinum crucible and cover are cleaned and briefly ignited. After cooling to room temperature, the crucible is transferred to the pan of the balance while the cover remains in the desiccator. The crucible is weighed and then placed on a sheet of clean paper. The calculated amount of limestone is placed on the bottom of the crucible, which is then returned to the pan and weighed again. If a microchemical balance is used, from 10 to 15 mg of limestone should be taken.

A quantity of anhydrous sodium carbonate, approximately equal to ten times the weight of the sample of limestone, is weighed on a clean piece of cardboard by means of a horn pan balance and then added to the limestone in the platinum crucible. The substances are mixed with the firepolished end of a glass rod of 1-mm diameter, which is finally dusted off into the crucible by means of a clean soft brush.

The crucible is placed inclined at an angle of 45 degrees on the triangle, and its sides are lightly tapped so that the contents collect at the lowest point. After covering with the platinum lid, the point of a small, non-luminous Bunsen flame is played for 5 minutes on the lowest portion of the crucible. The heating is continued for 2 more minutes by means of a small cone of blast flame.

**SEPARATION OF SILICIC ACID.** The platinum lid is removed from the crucible, and its underside is inspected with the aid of a magnifying glass for particles of the melt. If the lid is found to be clean, it is laid aside.

After it has cooled to room temperature, the crucible is placed on a sheet of clean paper, and the glass bulb, Fig. 75, is placed over the opening of the crucible. The contents of the crucible are to be treated with such a volume of 6 *M* hydrochloric acid that 0.06 ml of the acid is taken for each milligram of limestone. If some of the melt has been found on the lid, drops of the acid (0.03 ml approximately) are placed on the lid and from there transferred to the crucible. The acid is added, drop by drop, to the side of the glass bulb, which is slightly lifted for this purpose, and then allowed to flow via the projection to the bottom of the crucible. The bulb should be placed over the opening of the crucible before the drop of acid reaches the melt, and it should not be lifted up before the evolution of gas has come to an end. The rinsing of the lid should be complete with the fifth drop of acid. If any precipitate seems to adhere to the lid, it may be transferred to the beaker by applying alternately 2 drops of ethyl alcohol and 2 drops of the acid.

When all the acid has been added, the crucible is transferred to the steam bath, Fig. 75, and heated until the evolution of carbon dioxides is complete. The bulb is then rinsed into the crucible by means of 6 drops of distilled water and laid aside. The contents of the crucible are evaporated and finally baked as directed in Expt. 68. The baked residue is treated with 0.01 ml 6 *M* hydrochloric acid for each milligram of limestone. Care is taken that all the residue is moistened with the acid, which is then diluted by adding 0.1 ml of distilled water for each milligram of sample. The crucible is heated on the steam bath while the filterstick is connected to the siphon and tested.

**FILTRATION.** The paper cylinder in the head of the filterstick is moistened with a few drops of water before the filterstick is connected to the siphon. To test the filter, it is immersed in distilled water, and slight suction is applied. The rate of filtration should be approximately the same as that obtained with asbestos mats in filtersticks.

The filtration is performed as usual. The filtrate is removed completely, and care is taken that as little precipitate as possible is taken up by the glass part of the filterstick. Washing with four 0.5-ml portions of 6 *M* hydrochloric acid is followed by washing with six 0.5-ml portions of distilled water. Filterstick and crucible are rinsed with each portion of wash liquid, and each time the crucible is placed for 1 minute on the steam bath before the wash liquid is drawn off.

Finally, one washes the hands carefully, and then removes the filterstick from the siphon. While the head of the filterstick is held over the crucible, the paper cylinder is grasped with platinum-tipped forceps and thrown into the crucible. Then one rectangular piece of filter paper is picked up with the fingers and slightly moistened with distilled water. Outside and opening of the head of the filterstick are wiped to remove all silicic acid, and the rectangular piece is placed in the crucible. The second rectangular piece of filter paper is likewise used to wipe the tips of the forceps, and it too is then placed in the crucible. Forceps of stainless steel may be used in place of platinum-tipped forceps. If no suitable forceps are available, the first rectangular piece of filter paper is used to grasp the paper cylinder while it is removed directly with the fingers.

**ASHING AND IGNITION.** The crucible is placed inclined on the triangle and heated with a small non-luminous Bunsen flame. The heating must be started very slowly to dry the contents of the crucible previous to the ashing of the paper. After the charcoal has been completely oxidized, the crucible is placed on an aluminum heating block

or on a rectangular piece of nickel sheet which is supported by a ring stand.

One drop of concentrated sulfuric acid (0.03 ml) is introduced into the crucible and deposited close to the bottom so that it moistens the contents. The temperature of the heating block is gradually raised to 300° C so as to completely expel the sulfuric acid without spattering. (If the nickel sheet is used, the heating is started some distance away from the crucible.) Finally, the crucible is placed for 10 minutes in an electric furnace which has been heated to 850° C. Then the crucible is transferred to the triangle and left there to cool to approximately 100° C, whereupon it is transferred to the cooling block of the desiccator. After standing for 15 minutes the crucible is weighed. The ignition at 850° is repeated until the weight remains constant within  $3a_s$ . (The last weight is used in the calculation.)

**VOLATILIZATION OF SILICA.** The contents of the crucible are treated with 0.5 ml of 1 *M* sulfuric acid and then with approximately the same volume of hydrofluoric acid. The volume of the hydrofluoric acid is estimated on the lid of the platinum crucible before the acid is transferred to the crucible. The evaporation of the acids is performed under a hood. The heating is performed by means of either the heating block or the nickel sheet. The dry residue is ignited in the electric furnace for 5 minutes at 850° C, and then the crucible is weighed. The difference of the last two weighings corresponds to the weight of the silica.

**Notes.** The result of the microdetermination should agree with that of reliable macrodeterminations within  $\pm 0.2$  per cent  $\text{SiO}_2$ . The treatment with hydrofluoric acid may be repeated, if there is any doubt as to the complete elimination of the silica by the first treatment. If a blast lamp is used instead of an electric furnace, the times given for the various periods of ignition should be cut in half.

A blank may be carried out to determine the amount of residue left after evaporation of the hydrofluoric acid mixture used in the volatilization of the silica. Volatilization of platinum will hardly contribute an error of significance, since only a change in weight caused by the last short period of ignition after the volatilization of the silica has an influence on the result of the determination. Correction for the error introduced by the ash of the filter paper is of doubtful value if the percentage of silica in the ash of the paper is not known.

APPARATUS FOR ELECTRODEPOSITION <sup>2</sup>

The manipulative technique required in the isolation of weighing forms by electrodeposition is nearly as simple as that employed in residue determinations. While residue determinations do not require any mechanical transfer of matter, the mechanical separation of the electrolytic precipitate from the electrolyte does not present any difficulties, for it forms a smooth, tightly adhering coating on one of the electrodes. It is not surprising, therefore, that electrolytic determinations as well as residue determinations so far have always been among the first procedures tried, whenever gravimetric analysis has been applied to a new small scale.<sup>31, 32, 33, 34, 35</sup>

It is known that electrolytic separations are by no means as satisfactory in practice as might appear from a very elementary consideration of the theoretical background. Side reactions are prone to prevent complete precipitations, and foreign matter is frequently occluded by the precipitates. The application to microanalysis offers two additional difficulties. The factor  $f$  is usually equal to unity, since the determined metals are weighed as such. Consequently, weighing errors enter the result without reduction. Then, the liberation of gases during the electrolysis usually produces a very stable spray which is likely to carry away significant quantities of matter.

The apparatus may be very simple. A platinum crucible as cathode and a platinum wire as anode will often do. Nevertheless, a great variety of designs is used<sup>2</sup> for the sake of efficient operation. The apparatus shown in Fig. 76 is a modification of that used by Pregl.<sup>14</sup> The electrolysis is carried out in a Pyrex test tube of 16-mm diameter and 105-mm length. The cathode consists of a cylinder of platinum gauze of 10-mm diameter and 30-mm height, to which is welded a platinum wire of 1-mm diameter. The end of the wire is bent to a hook so that the cathode may be suspended above the pan of the balance from the hook attached to the stirrup. The gauze cylinder is usually reinforced at base and top by strong wires which are welded to the gauze before it is bent into cylindrical shape. Three small drops of Pyrex glass may be fused at equal distance from one another on each of these two reinforcing wires. If the beads are attached to

<sup>31</sup> W. Nernst and E. H. Riesenfeld, *Ber.*, 36, 2086 (1903).

<sup>32</sup> E. Jänecke, *Z. anal. Chem.*, 43, 547 (1904).

<sup>33</sup> E. H. Riesenfeld and H. F. Möller, *Z. Elektrochem.*, 21, 137 (1915).

<sup>34</sup> F. Emich in *Abderhalden's Handbuch der biologischen Arbeitsmethoden*, Abt. I, T. 3, p. 268, Vienna and Berlin, Urban u. Schwarzenberg, 1921.

<sup>35</sup> E. Wiesenerger, *Mikrochemie*, 10, 10 (1931).

base and top of the cylindrical electrode so that they point outward, the metal of the electrode can easily be prevented from touching the walls of the test tube when the electrode is inserted or removed. The beads are obtained on the electrode by touching the wire with the

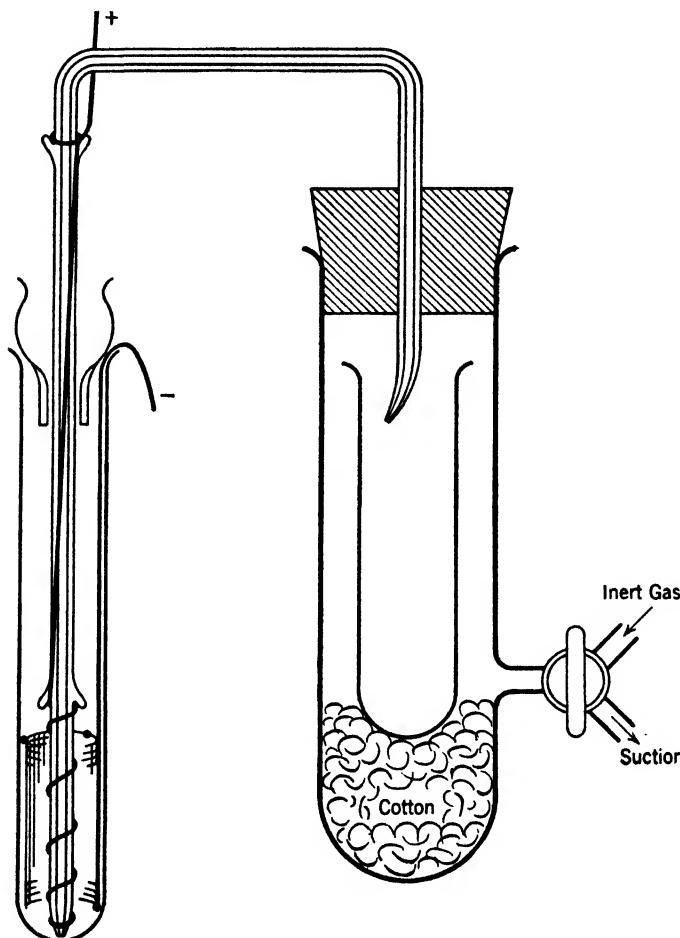


FIG. 76. Electrodeposition.

molten end of a Pyrex rod of approximately 1-mm diameter. Most of the glass is immediately drawn off the wire, and the rest is fused to a round bead by heating platinum and glass. Whenever cracks develop in the glass, which might lead to inclusion of electrolyte, the beads are repaired by igniting them with the electrode.

The anode consists of a platinum wire 0.5 mm in diameter, which is wound in the form of a helix around the lower part of the ascending

arm of the siphon. The siphon is bent from Pyrex tubing of 2-mm bore. The two pairs of glass horns serve to keep the anode in its proper position. The stem of the small funnel is wide enough to permit sliding the funnel up and down along the siphon. The wide suction tube contains a microbeaker for the collection of electrolyte and washings. The three-way stopcock allows the suction tube to be connected with either a supply of inert gas or with the vacuum line.

The electric current may be furnished by a storage battery which gives 4 volts. A variable resistance of 50 to 100 ohms is inserted in series with the electrolytic cell, and a voltmeter is hooked up parallel to the cell. Connection with the electrodes is best made by means of small clips which grasp the wires with smooth, polished surfaces at the points indicated in the figure by plus and minus signs.

## EXPERIMENT 70

### Determination of Copper and Nickel in German Silver<sup>36</sup>

German silver : 52 to 64 per cent copper.

11 to 25 per cent nickel.

Copper :  $100f = 100$  per cent Cu.

Nickel dimethylglyoxime :  $100f = 20.31$  per cent Ni.

$\log 100f = 1.30779$  (for the determination of nickel).

Mass of sample,  $S = 800a_g^3$

Weighing forms dried at approx. 80° C (Cu) and at 120° C (NiC<sub>8</sub>H<sub>14</sub>N<sub>4</sub>O<sub>4</sub>), respectively.

#### Required:

Apparatus for electrolysis described above. Electrolytic beaker cleaned with chromic-sulfuric acid.

Weighing tube, Fig. 77.

Quartz beaker of 15-ml capacity, glass bulb shown in Fig. 75.

Microbeaker and filterstick with asbestos mat, ready for drying.

Supply of pure inert gas (hydrogen, nitrogen, or carbon dioxide) of low pressure.

Gas wash bottle containing ammonia and a few pellets of sodium hydroxide.

Clean glass rod supported horizontally approximately 20 cm above table top.

German silver, filings, carefully mixed, extracted with acetone, and dried.

16 M nitric acid, c.p.

18 M sulfuric acid, c.p.

1 M sulfuric acid.

6 M hydrochloric acid, c.p.

Ammonium citrate, solid.

Dimethylglyoxime, cold saturated solution in 95 per cent ethyl alcohol.

<sup>36</sup> A. J. Llacer, J. A. Sozzi, and A. A. Benedetti-Pichler, *Ind. Eng. Chem., Anal. Ed.*, 13, 507 (1941).

The alloy is dissolved in a mixture of sulfuric and nitric acids. The copper is separated by electrolysis and weighed on the electrode. The nickel is determined in the electrolyte by precipitation and weighing of the inner complex with dimethylglyoxime.

**WEIGHING THE SAMPLE.** The weighing tube, Fig. 77, is easily made from a test tube of soft glass, which is first drawn out to a tube of approximately 3-mm bore. The weighing tube is cut out of that part where the test tube tapers to the diameter of the tube. The wide opening of the tube should have a diameter of 6 to 8 mm. The tube is slightly bent as shown in the figure. The depression *c* is blown up, and handle *a* and legs *b* are attached. If the tube shall be used with

a microchemical balance, care must be taken to make the distance between the legs and the part *d*, on which the tube rests, short enough to fit the small pan of the balance. The weighing tube is cleaned, dried, wiped with flannel and chamois, allowed to acquire room temperature, and then kept inside the balance case together with a suitable glass tare.

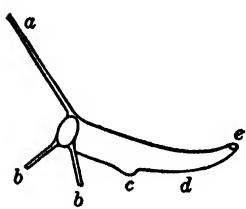


FIG. 77. Weighing tube, approximately half natural size.

The weighing tube is first weighed empty. Its handle *a* is then grasped with cork-tipped forceps, and the tube is placed on a sheet of clean paper. The calculated amount of sample is introduced by means of a spatula so that it collects in the depression *c*. From 3 to 5 mg of the alloy should be taken when working with a microchemical balance. The tube is weighed again and then inserted, opening *e* first, into the electrolytic beaker which is held inclined at an angle of 30 degrees to the horizontal. When the electrolytic beaker is swung upright, the weighing tube remains suspended at the opening by its handle and legs. Tapping the outside of the beaker will cause the filings of the alloy to drop to the bottom of the beaker. If necessary, the interior of the weighing tube may be rinsed with some water from the wash bottle.

**SOLUTION OF THE ALLOY.** The liberation of nitrogen oxides during the treatment with acids may cause considerable losses, if one does not guard against the escape of spray from the beaker. Thus, the alloy is first covered with 0.05 ml of distilled water, and a glass bulb, Fig. 75, is placed on the opening of the beaker. Then while the bulb is slightly lifted 16 *M* nitric acid is added drop by drop from a pipet so that it flows down along the projection of the bulb and along the wall of the beaker. Altogether, 3  $\lambda$  of the nitric acid is taken for each milligram of alloy. If necessary, solution is hastened by gentle heating with a small flame so that the evolution of nitrogen oxides never

becomes violent. Finally,  $2\lambda$  of 18 *M* sulfuric acid is added for each milligram of alloy, and, if necessary, heating is continued until the solution of the alloy is complete. When the liberation of nitrogen oxide ceases, bulb and electrolytic beaker are rinsed down with 0.5 ml of distilled water, and the bulb is removed. The solution is heated for 5 minutes on the steam bath, and air is blown into the beaker from time to time to remove the nitrogen oxide given off by the solution. The electrolytic beaker is finally set aside and covered.

**CLEANING OF ELECTRODES.** The wire gauze electrode is placed in a short test tube which is half filled with concentrated nitric acid. The test tube is heated on the steam bath for a few minutes, and in the meantime two beakers of 400-ml capacity, numbered 1 and 2, are filled with distilled water and covered with watch glasses. The electrode is first carefully rinsed with tap water, and then twice with distilled water, the water of beaker 1 always being used first and that of beaker 2 last. The excess of distilled water is removed by shaking the electrode. Holding the end of the wire with clean fingers, the gauze cylinder is thoroughly ignited by holding it in the point of a large, non-luminous Bunsen flame. After igniting the gauze part, the glass beads are inspected for cracks with the aid of a magnifying glass. Then the lead-in wire is grasped with platinum-tipped forceps close to the gauze cylinder, and the whole wire is ignited. The electrode is then hung on a horizontally held glass rod for cooling. After 5 minutes the electrode is picked up with forceps and suspended from the hook attached to the left-hand stirrup of the balance. The electrode may be counterbalanced with pieces of the set of weights, which are best left on the right-hand pan in readiness for the weighing of the copper deposit. If the weights are removed between the two weighings, care must be taken that exactly the same pieces of the set are used for counterbalancing in the second weighing. The ideal tare would be a spiral of heavy platinum wire. After weighing, the electrode is removed from the balance with forceps. From that point to the second weighing the end of the lead-in wire may be handled with clean fingers.

**DEPOSITION OF COPPER.** The electrolytic beaker containing the solution of the alloy is clamped into a stand so that the bottom of the beaker is approximately 15 cm above the table top, and the wire gauze cathode is inserted so that it rests with the glass beads on the bottom of the beaker. One drop of ethyl alcohol is added to decrease the stability of the spray,<sup>37</sup> and then sufficient 1 *M* sulfuric acid to

<sup>37</sup> A. A. Benedetti-Pichler, *Z. anal. Chem.*, **62**, 321 (1923).

bring the meniscus of the electrolyte nearly up to the top of the gauze cylinder.

A microbeaker of 15-ml capacity, made of clear fused quartz, is treated with cleaning solution, rinsed with water, and then introduced into the special suction tube belonging to the electrolytic apparatus. The suction tube is clamped in a stand so that the free end of the siphon is at the same height as the bottom of the electrolytic beaker. A slow current of inert gas is sent through suction tube and siphon, and then the leg of the siphon with the wire anode is introduced into the electrolytic beaker as shown in Fig. 76. The rate of gas flow is immediately adjusted to obtain approximately 1 bubble in 3 seconds emerging from the tip of the siphon which touches the bottom of the beaker. This current of gas is necessary to prevent electrolyte from entering the siphon before the precipitation of the copper is complete. The stirring effect must not be overrated, since the stream of gas must be so slow that it cannot carry away a significant amount of the spray produced during electrolysis.

The small funnel is made to rest on the opening of the electrolytic beaker, and the space between the stem of the funnel and the siphon is sealed by adding a drop of water. After making the electric connections, the electrolyte is heated to approximately 70° C with a small Bunsen flame. The flame is then removed, and the electrolysis is started with 2.7 to 3.1 volts across the electrodes. Approximately 5 minutes after appearance of the first copper deposit, the color of the electrolyte will indicate that most of the copper has been precipitated. While the electrolysis is continued, the electrolyte is heated to boiling, and the ring of condensate is allowed to rise close to the opening of the beaker. After removal of the flame, beaker and funnel are rinsed with a few drops of water. The electrolysis is continued for 10 more minutes, and during the second half of this period the electrolyte is cooled by raising a beaker with cold water so that the lower portion of the electrolytic beaker is immersed. The resistance is continually adjusted so as to prevent the emf from exceeding 3.1 volts.

**COLLECTION OF THE ELECTROLYTE.** Without interrupting the current, the electrolyte is completely siphoned off by the application of *light* suction. The suction is then interrupted, and electrolytic beaker and funnel are rinsed with distilled water until the gauze cylinder of the cathode is again completely immersed. A slow current of inert gas is sent through the wash liquid for 2 minutes, after which the wash liquid is siphoned off with light suction. The washing is repeated twice in exactly the same manner. Finally, the suction is interrupted, and the wire gauze is again covered with distilled water.

Siphon and anode are removed first, and immediately afterwards the cathode, which is quickly placed in beaker 1 containing distilled water. Now, and not before, the electric current is shut off.

**WEIGHING OF THE CATHODE WITH THE COPPER DEPOSIT.** The cathode is briefly rinsed in both batches of distilled water held ready for this purpose. The excess of water is removed by lightly shaking the electrode, which is then dried by moving it fanwise approximately 30 cm above a non-luminous Bunsen flame. The cathode, which has been held so far with clean fingers at the hook-shaped part of the lead-in wire, is now grasped with platinum-tipped forceps near the gauze cylinder but sufficiently far away from it so that the forceps do not get in contact with the copper deposit. Now the end of the lead-in wire, which has been handled with fingers, is ignited for a few seconds. The electrode is hung on the glass rod to acquire room temperature and weighed after 5 minutes.

The copper deposit should be light in color and of metallic appearance. It is removed from the electrode by following the instructions given for the cleaning of the electrode.

**DETERMINATION OF NICKEL.** The quartz beaker is removed from the suction tube of the electrolytic apparatus and placed on the steam bath. Air is blown into the beaker, and its contents are concentrated to a volume of 1 or 2 ml. The time is utilized for drying, wiping, and weighing the microbeaker and filterstick intended for the determination of nickel.

The transfer of the concentrated electrolyte to the microbeaker is performed by means of the suction device shown in Fig. 15A. Very light suction must be used. The transfer is made complete by rinsing with five 0.3-ml portions of distilled water. Each portion of wash liquid is made to wet the whole interior surface of the quartz beaker, and after brief whirling in a centrifuge it is sucked off.

The contents of the microbeaker are treated with 5  $\lambda$  of 6 *M* hydrochloric acid and with approximately 0.2 mg of solid ammonium citrate for each milligram of alloy. After the addition of a small drop of methyl red solution, the contents of the beaker are mixed by swirling from time to time while air laden with ammonia is blown on the surface of the solution until the color of the solution changes to yellow. The alkaline solution must remain perfectly clear. It is diluted with distilled water to a volume of approximately 4 ml (6 ml if more than 20 mg of alloy have been taken for analysis).

The solution is now heated on the steam bath, and very small drops of dimethylglyoxime solution are added from a reagent pipet with fine capillary tip. After the addition of each drop of reagent, the

contents of the beaker are mixed. The red precipitate settles to the bottom of the microbeaker, and it is easy to recognize when the precipitation is complete. Then, 3 drops of the reagent are added in excess, and a volume of distilled water is added which is equal to the volume of the total amount of oxime reagent employed. The beaker with the precipitate is left for 10 minutes on the steam bath. It is then placed under a bell jar and allowed to stand for 30 minutes.

Filtration is followed by washing with four 1-ml portions of hot water. Each time, the walls of the beaker are washed down with hot water from the wash bottle. Beaker and contents are heated on the steam bath for 1 minute, and then the hot wash liquid is completely sucked off through the filterstick. Drying at 120° C in the drying tube requires 10 minutes.

**Notes.** The deviation of the percentages found for copper and nickel should agree with the results of reliable macrodeterminations within the limits  $\pm 1.2$  per cent copper and  $\pm 0.5$  per cent nickel. If larger discrepancies persist in spite of careful work, and also the results of different microdeterminations deviate from one another to a greater extent than that indicated by the above given limits, heterogeneity of the alloy may be the cause. Satisfactory results may then be obtained by dissolving a large sample and performing the determination on a small aliquot portion of the solution.<sup>36</sup> Of course, the amount of alloy contained in the aliquot must approximately agree with the mass of sample  $S$  calculated from the precision of the balance used.

### (b) GAMMA PROCEDURES

Quantitative determinations on samples a few gammas in mass require a precision of weighing of at least 0.01 γ, which up to the present could not be obtained with beams of metal. The use of refined analytical balances is therefore out of the question, and only beams made entirely of clear fused quartz have been employed with success in work of this kind.

Such quartz balances, often called "microbalances," have always been made, and frequently designed, by the experimenter who used them. Of course, the case, which usually consists of heavy metal sheet with a few small glass windows, the column or a substitute, and parts of the arresting mechanism may be supplied by a mechanic. In some models the bearings consist of knife edges, ground of clear fused quartz and fused to the beam. More frequently, the beam is supported by quartz fibers a few microns in diameter. Small quartz

hooks are usually attached to the beam by quartz threads which are made so fine at the point where they are attached to the beam that the flexibility of the thread eliminates the need for special terminal bearings. Small dishes of platinum foil, which usually serve as containers for samples and weighing forms, are suspended from the quartz hooks of the microbalances, and they may be considered integral parts of these balances which are commonly adjusted to give the zero readings with the empty dishes on the hooks.

Weights in the usual sense are not used. The relative mass of the objects of investigation is obtained in one of the following three ways: (1) observation of the displacement of the point of rest; (2) measuring the change of buoyant effect required to return the beam to the original point of rest; and (3) determination of the strength of electric current required to return the beam to its original point of rest by electromagnetic compensation. The second principle makes it necessary to change the gas pressure inside the balance case, an operation which may cause difficulties in analytical work. Of course, electromagnetic compensation should not be used for the weighing of ferromagnetic substances.

The maximum load which can be carried by these quartz balances is of the order of a few tenths of a gram, and the dimensions of apparatus which is to be weighed are severely limited. If it is further considered that quantitative transference of a few gammas of material must offer serious difficulties, it is not surprising that gravimetric work has not progressed beyond residue determinations<sup>34, 35</sup> and electrodeposition methods.<sup>35</sup>

Anybody wishing to work in this particular field must become thoroughly acquainted with the construction of microbalances. The required information has been compiled by Emich,<sup>34</sup> and some highly interesting sidelights on the polishing of knife edges on quartz rods may be found in an address by Hartung.<sup>38</sup> It should be pointed out, however, that efficient work with highly sensitive microbalances requires a location some distance removed from railroads and heavy traffic lines. The balances are quite sensitive to vibrations, and they should be mounted on pillars coming up from the foundation of the building. Maintenance of an even temperature in the balance room is desirable, and proximity to electric machines and transmission lines must be avoided with balances using electromagnetic compensation.

<sup>38</sup> E. J. Hartung, *Presidential address, Rep. Australian New Zealand Assoc. Advancement Sci., Brisbane Meeting, May-June, 1930.*

## B. TITRIMETRIC DETERMINATIONS<sup>1</sup>

A general discussion of microtitrimetry is best started with a brief review of the corresponding macrotechnique. The customary measuring buret has a capacity of 50 ml, and if the graduated portion is given a length of 50 cm, the graduation marks corresponding to 0.1 ml will lie 1 mm apart. Millimeter scales can be easily read with a precision of  $\pm 0.1$  mm, and one may be reasonably certain that the deviation caused by two readings of the buret will hardly ever exceed  $\pm 0.2$  mm in length or  $\pm 0.02$  ml in volume. Of course, it is not advisable to try using the buret to its total capacity, but a skillful operator will arrange to use approximately 40 ml of standard solution in a titration so as to get the benefit of a maximum of precision. The relative error  $\rho'$  produced by reading two positions of the meniscus will then be

$$\rho' = 1000 \frac{\pm 0.2}{400} = \pm 0.5 \text{ part per 1000}$$

Since the normality  $N_{\text{macro}}$  of the standard solutions usually has values between 1 and 0.1, the 40 ml of standard solution contains from 4 to 40 mg-vals<sup>2</sup> of active reagent, and an equal number of milligram-equivalents of constituent to be determined may be titrated. This corresponds to approximately 0.1 to 1 gram of determined constituent and 1 to 10 grams of sample, if the sample contains 10 percent of the determined constituent.

It seems reasonable to define the microprocedures as follows:

Centigram procedures: 1 to 10 cg of sample.  
40 to 400  $\gamma$ -vals of determined constituent.

Milligram procedures: 1 to 10 mg of sample.  
4 to 40  $\gamma$ -vals of determined constituent.

Gamma procedures: 1 to 10  $\gamma$  of sample.  
4 to 40 my-vals of determined constituent.

<sup>1</sup> A comprehensive discussion of milligram procedures of titrimetry has been given by J. Mika, *Die exakten Methoden der Mikromassanalyse, Die chemische Analyse*, Vol. 42, Stuttgart, F. Enke, 1939.

<sup>2</sup> The abbreviations mg-val,  $\gamma$ -val, and my-val stand for milligram-equivalent, gamma-equivalent, and milligamma-equivalent.

With microburets, it is advisable to retain millimeter rulings, if the position of the meniscus is to be read either with the unaided eye or with the help of a magnifying glass. This, in turn, determines the length of the calibrated part of the buret as a function of the required precision  $\rho'$ . Since the uncertainty introduced by reading two positions of the meniscus is still  $\pm 0.2$  mm, the useful length  $l$  of the calibrated part of the microburet must be:

$$l = \frac{200}{\rho'} \text{ mm}$$

or 400 mm, if the same precision is required which is obtainable with the macroburet. This condition is not affected by the choice of a particular size of microsample, and it holds equally for macroburets having millimeter rulings.

After the length of the calibrated portion of the buret has been determined by deciding on precision and rulings, the bore of the calibrated portion of the buret is determined by the size of the sample and by the normality  $N_{\text{micro}}$  of the standard solution used.

Starting with the equation for the volume of a cylinder, one derives for the diameter  $d$  of the bore of the calibrated part of the buret:

$$d = \sqrt{\frac{4c}{\pi l}} \text{ mm}$$

if the capacity  $c$  of the useful part of the buret is substituted with the dimension  $\lambda$ , and the length  $l$  in millimeters. Provided that in microanalysis a standard solution of the same normality as in macrotitration is used, the required capacity  $c$  is derived from the capacity of the macroburet ( $40 \text{ ml} = 40,000 \lambda$ ) by multiplication by the factor: size of microsample over size of macrosample. If, however, a more dilute standard solution is used in microtitration, the dilution factor,  $\phi = N_{\text{macro}}/N_{\text{micro}}$ , must be taken into consideration:

$$c = 40,000 \phi \frac{S_{\text{micro}}}{S_{\text{macro}}} \lambda$$

Substitution gives the diameter of the bore of the calibrated part of the buret as a function of precision determined by the error in reading the buret,  $\rho'$ , dilution factor,  $\phi$ , and reduction of the size of sample,  $S_{\text{micro}}/S_{\text{macro}}$ :

$$d = 16 \sqrt{\rho' \phi \frac{S_{\text{micro}}}{S_{\text{macro}}}} \text{ mm}$$

Table V is a compilation of the dimensions of the bore needed in centigram, milligram, and gamma procedures. In addition, the useful length of the calibrated portion of the buret is listed as a function of the desired precision of reading. The heavy frames indicate the conditions which permit dispensing with the use of microburets. An inner diameter of 11 mm corresponds to that of a macroburet. Of course, it would be foolish to employ burets of 16-, 35-, or 50-mm inner diameter.

TABLE V

DIAMETER OF THE BORE OF A BURET AS FUNCTION OF PRECISION, MASS OF SAMPLE, AND DILUTION OF STANDARD SOLUTION

$\pm \rho'$  = relative error in parts per thousand of the volume of standard solution caused by errors in reading the position of the menisci.

$l$  = lengths, in millimeters, of the part of the buret actually used.

$c$  = capacity of the same part of the buret.

$\phi$  = dilution factor = normality of standard solution in the macrotitration over normality of standard solution in microtitration.

It is assumed that all burets are provided with millimeter scales, and that the total error committed in reading two positions of the meniscus does not exceed  $\pm 0.2$  mm. The diameter of the bore is given in millimeters.

$\pm \rho'$ parts per 1000	$l$ in mm	$S = 1$ to $10$ cg 40 to 400 $\gamma$ -vals			$S = 1$ to $10$ mg 4 to 40 $\gamma$ -vals			$S = 1$ to $10$ $\gamma$ 4 to 40 m $\gamma$ -vals		
		$\phi = 1$ $c = 0.4$ ml	$\phi = 10$ $c = 4$ ml	$\phi = 100$ $c = 40$ ml	$\phi = 1$ $c = 0.04$ ml	$\phi = 10$ $c = 0.4$ ml	$\phi = 100$ $c = 4$ ml	$\phi = 1$ $c = 0.04$ $\lambda$	$\phi = 10$ $c = 0.4$ $\lambda$	$\phi = 100$ $c = 4$ $\lambda$
0.5	400	1.1	3.5	11	0.35	1.1	3.5	0.011	0.035	0.11
5	40	3.5	11	(35)	1.1	3.5	11	0.035	0.11	0.35
10	20	5	16	(50)	1.6	5	16	0.05	0.16	0.5

The use of ordinary macroburets is made possible in these instances by the employment of very dilute standard solutions and by relaxing the precision requirements. Of these two measures, the first is frequently objectionable, for it leads to an undesirable dilution of the titrated liquid. The use of 40 ml of standard solution in centigram procedures would be like using 4 liters of standard solution for the titration of 1 gram of sample. A survey of the theory of titrimetric methods will show that the accuracy of end-point indication is best when working with moderately concentrated solutions. A notable exception is furnished by potentiometric redox titrations. Table V shows that quite narrow capillaries are required for the calibrated part of the burets if the standard solutions are to be used in the same

concentrations as in macrotitration, but it is definitely advisable to spend the *additional effort in construction and use of such burets than to accept the disadvantages of large indicator corrections*. If necessary, the precision of the reading of the meniscus may be improved, which then permits use of shorter burets of larger bore.

Concerning the titrated solution, it may be stated that the concentrations of determined substance and indicator should be the same as in macrodeterminations. This calls for reduction of the volume in proportion with the decrease of the amount of sample.

$$v_{\text{micro}} = v_{\text{macro}} \frac{S_{\text{micro}}}{S_{\text{macro}}}$$

Frequently it will be advisable to make the volume of the titrated solution approximately half of the useful capacity of the buret: 0.5c.

### VOLUMETRIC FLASKS

Volumetric flasks are used to prepare a definite volume of solution from a given amount of material. The purpose of the operation is either the preparation of a standard solution of a known substance or the aliquot partition of the material under investigation. Standard solutions are usually made in relatively large batches, and the customary volumetric flasks of 10- to 1000-ml capacity will be found useful in microanalysis. Aliquot partition is frequently performed immediately after dissolving the sample so as to get with a minimum of effort a large number of solutions which may be treated simultaneously. If the material under investigation is very heterogeneous, the analyst may be forced to dissolve a quite large sample to obtain results representing the average content, and only a small part of the solution will then be taken for analysis. This procedure must be more frequently employed in microanalysis than in macrodeterminations, since a degree of heterogeneity which would hardly be noticed when working with samples of 1-gram mass may lead to serious inconsistencies if samples of 1-mg mass are used.

A statistical investigation<sup>8</sup> has shown that a sample taken from a mixture of solids should contain about one million particles of approximately identical size in order to represent closely the composition of the material in question. There is no doubt that determinations can be performed with success on samples a few milligrams in mass if the material can be properly mixed and ground to a fine

<sup>8</sup> B. Baule and A. A. Benedetti-Pichler, *Z. anal. Chem.*, 74, 442 (1928).

powder so that the diameter of the particles does not exceed 10  $\mu$ . Frequently it will be found preferable, however, to dissolve a large sample and to carry out microdeterminations on small fractions of the solution of the sample.<sup>4, 5</sup> As a rule, volumetric flasks of 10- to 500-ml capacity and micropipets delivering 0.01 to 1 ml will then be used for the performance of aliquot partition. The advantages of this procedure are obvious. Its precision has been tested.<sup>6</sup>

The design of volumetric flasks of small capacity presents little difficulty. Caley<sup>7</sup> describes a form which facilitates the mixing of the contents. Holmes,<sup>8</sup> Krumholz,<sup>9</sup> and Mika<sup>1</sup> describe volumetric flasks of 1- to 10-ml capacity which are essentially tubes with a constriction at the graduation mark. These tubes may be centrifuged previous to the adjustment of the position of the meniscus. Krumholz uses a mark somewhat above and another somewhat below the mark designating the desired volume. The volume corresponding to each of these marks is determined. In use, careful adjustment of the meniscus is not required, for its position between the marks can be estimated with sufficient accuracy.

### PIPETs

Pipets of 0.5-, 1-, and 2-ml delivery are generally useful in quantitative microanalysis. In the measuring of samples for microtitration, pipets delivering 0.02, 0.2, and 2 ml appear desirable as indicated by the values for  $c$  in Table V. As to the construction, one provision applies to all types of pipets: at the calibration mark the bore of the pipet should be so small that the reading error becomes entirely negligible. Mika<sup>1</sup> makes the reasonable stipulation that a reading error of  $\pm 0.3$  mm shall be equal to  $\pm 0.2$  part per 1000 of the capacity  $c$  of the pipet. If the capacity is stated in lambdas, the diameter of the bore at the calibration mark is given by the equation

$$d = 0.029 \sqrt{c} \text{ mm}$$

The opening at the tip of the pipets should be made so narrow that the meniscus travels through the capillary parts at a rate between 25

<sup>4</sup> A. A. Benedetti-Pichler, *Z. anal. Chem.*, **62**, 321 (1923).

<sup>5</sup> A. J. Llacer, J. A. Sozzi, and A. A. Benedetti-Pichler, *Ind. Eng. Chem., Anal. Ed.*, **13**, 507 (1941).

<sup>6</sup> A. A. Benedetti-Pichler, *Z. anal. Chem.*, **61**, 324 (1922).

<sup>7</sup> Earle E. Caley, *Ind. Eng. Chem., Anal. Ed.*, **13**, 204 (1941).

<sup>8</sup> F. E. Holmes, *Ind. Eng. Chem., Anal. Ed.*, **13**, 586 (1941).

<sup>9</sup> P. Krumholz, *Mikrochemie*, **25**, 242 (1938).

and 100 mm per minute when the pipet is emptied. If the pipet is a capillary without any kind of bulb, the rate should be decreased to 10 to 20 mm displacement of the meniscus per minute.

As a rule, no attempt is made to prepare pipets of exactly the nominal capacity. In consequence it is essential that every pipet be provided with a number so that it can be identified beyond doubt and its capacity found in the records.

It is obvious that pipets must be carefully freed from fat before they are calibrated, and that they must be equally clean whenever they are used. If the pigment used in the markings permits, the pipets may be continuously kept in a cylinder filled with cleaning solution. For rapid and efficient cleaning, a cold solution of permanganate in sulfuric acid may be used. This very dangerous agent is always freshly prepared in *very small* quantities and discarded immediately after use by pouring it into a large volume of water. The mouth must *never* be used for sucking this solution or any other corrosive reagent into an apparatus. The cleaning solution is prepared by placing 1 to 3 crystals of potassium permanganate in a *dry* test tube and adding 0.5 to 1 ml of concentrated sulfuric acid. A deep green solution is obtained, which reacts explosively when brought in contact with visible quantities of organic matter. At times, treatment with this cleaning solution leaves a brown deposit of manganese dioxide on the walls of the apparatus. The deposit is quickly removed by rinsing with an acid solution of ferrous-ammonium sulfate.

**Pipets Calibrated for Delivery. Transfer Pipets.** This type of pipet, Fig. 78A, is practical for the measurement of volumes of 0.1 ml and more. Suction is recommended for filling the pipet. Figure 78A illustrates this operation. A glass tube, which is connected at *a* by means of a long rubber tubing to the suction line, has an opening at *b* which is closed when suction is desired. A short piece of flexible rubber tubing of 2- to 3-mm bore is partly forced over the end *c* of the rather wide glass tube which makes the connection to the suction line. The free end of this rubber tubing fits the tapering portion near the bulb of the pipet.

The liquid is taken up a few millimeters above the calibration mark. The pipet is then removed from the suction device. The outside of the tip is wiped dry with filter paper. Then, while holding the pipet nearly horizontal, one removes the excess of liquid by repeatedly touching filter paper to the tip of the pipet. For draining, the pipet is held vertical, and the tip is made to touch the surface of the delivered liquid. The considerable amount of liquid which is

finally held in the delivery tube of the pipet by capillary attraction is expelled by closing the "mouthpiece" of the pipet and warming the bulb by touching it with the fingers. The liquid should be expelled slowly, and it is stripped off the tip by touching it to either the surface of the liquid or to the wet wall of the container.

The same procedure is followed when the pipets, which can be made by anybody with some experience in glass blowing, are calibrated by weighing the water delivered. It is understood that all the customary precautions must be observed, as substitution method of weighing, calibrated set of weights, correction for the buoyant effect (with special consideration of the density of the fractional weights), and correction for the expansion of glass. Distilled water of the temperature of the balance room is used, and special care must be taken to handle all apparatus by means of forceps or several layers of chamois to prevent the transfer of heat from the hands to the apparatus. Of course, a precise balance is required, and the effect of the average deviation of a single weighing on the precision of the calibration should be considered. If possible, the precision should be better than 1 part per 1000. Errors caused

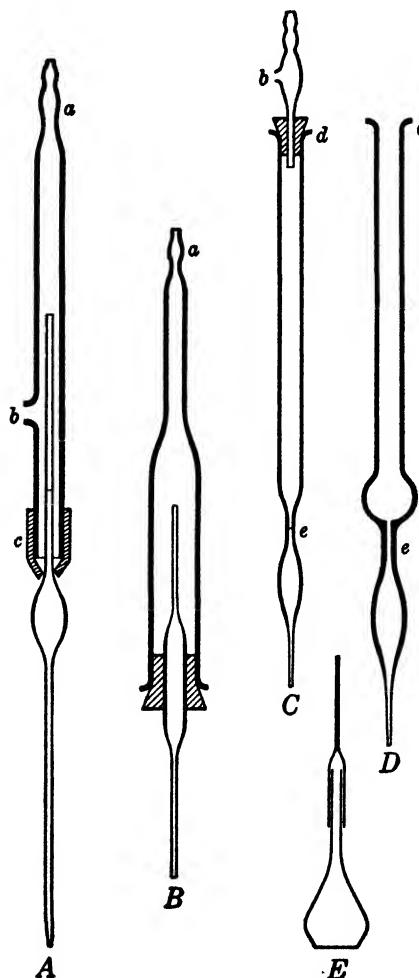


FIG. 78. Micropipets. *A*, transfer pipet; *B*, transfer pipet with automatic adjustment of the zero point; *C*, Pregl's wash-out pipet; *D*, wash-out pipet with automatic adjustment of the zero point.

by the evaporation of water during manipulations may be reduced by employing a small flask, Fig. 78*E*, with very narrow neck, which can be closed by sliding a snugly fitting tube over the neck.

*Transfer Pipets with Automatic Adjustment of the Zero Point.*<sup>10, 11, 12</sup> Pipets of this kind, Fig. 78B, are mounted in a glass tube which may be connected through *a* to suction and controlled pressure. They are especially suited for the transfer of small volumes, 0.2 ml to 1  $\lambda$ , and, of course, they may be given the form of a straight capillary without bulb.

For filling, the pipet is inclined at an angle of 30 to 45 degrees to the horizontal. The liquid is sucked up until it reaches the fine capillary above the bulb. Then the suction is broken, and the solution rises by capillary attraction to the opening of the fine capillary. The pipet is withdrawn in the inclined position, and liquid adhering to the outside of the tip is removed with filter paper before the pipet is returned to the vertical position for delivery. Emptying requires the application of well-regulated pressure to assure the proper rate of flow. Small pipets of this type fill automatically when the tip is touched to a solution. They may be kept continuously in the perpendicular position.

The directions for calibration apply as given for simple transfer pipets. It may be pointed out, however, that the following technique of calibrating may prove more satisfactory with pipets of small capacity: The pipet is filled with water, which is then expelled as usual so as to leave the proper amount of water on the interior surface. The pipet is rapidly enclosed in a weighing tube and weighed. It is then filled with water and weighed again. The difference between the two weighings represents the weight of water delivered by the pipet.

**Pipets Calibrated for Capacity.** The wash-out pipets, Fig. 78C and D, which have been introduced into the microchemical technique by Pregl,<sup>13</sup> are calibrated for capacity by weighing them filled with mercury. Because of the high density of mercury, the effect of the weighing error is reduced to approximately one-fourteenth, and volumes from 10  $\lambda$  up may be determined with a high degree of reliability.

The pipets are filled in the usual way. Figure 78C shows how connection to a suction line may be obtained. The adjustment of the zero point is automatic with the type shown in Fig. 78D.<sup>14, 15</sup> The pipets are emptied by blowing with the mouth. To collect the con-

<sup>10</sup> K. Linderstrøm-Lang and H. Holter, *Z. physiol. Chem.*, 201, 9 (1931).

<sup>11</sup> K. Schwarz and C. Schlösser, *Mikrochemie*, 13, 18 (1933).

<sup>12</sup> E. Abel and F. Fabian, *Mikrochim. Acta*, 1, 43 (1937).

<sup>13</sup> F. Pregl, *Die quantitative organische Mikroanalyse*, 1st ed., Berlin, J. Springer, 1917.

<sup>14</sup> E. Corneel and E. Cottet, *Bull. soc. chim.*, 35, 682 (1924).

<sup>15</sup> M. Nicloux, *Bull. soc. chim. biol.*, 7, 753 (1925).

tents quantitatively, the pipets are then rinsed with three or four portions of wash liquid of  $c/4$  volume, which are introduced through the mouthpiece  $d$  of the pipet. Of course, this approximately doubles the volume of the solution taken, but frequently it is possible to use for rinsing the reagent solutions which are next to be added.

For cleaning of volumetric apparatus, the gadgets shown in Figs. 14B and 78A are valuable aids.

### BURETS

The microburets, which will now be discussed, rely mainly upon gravitational force and surface tension for the regulation of the out-

flow of standard solution. In the introductory section, Table V, it was shown that it is advisable to use capillaries of 0.01- to 3.5-mm bore for the calibrated part of the burets. In titration, the necessity of getting a very slow rate of travel for the receding meniscus requires selection of a still finer bore for the tips of the burets. Consequently, the surface forces existing in microburets must be of considerable strength, and it seems natural to utilize them.

It is known that a liquid rises in a capillary to a height  $h_1$ , Fig. 79, which depends upon the diameter of the capillary at the level I of the meniscus, the density, and

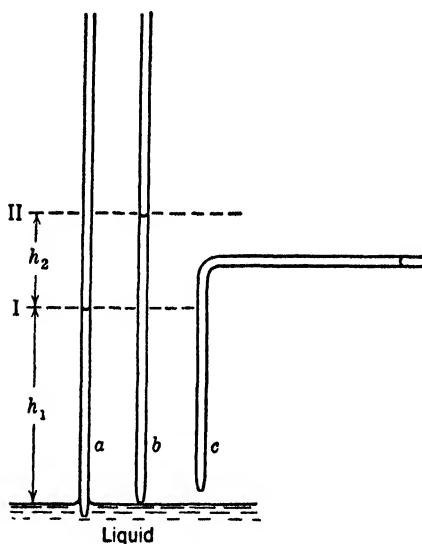


Fig. 79. Surface forces in capillaries.

the surface energy of the liquid. It is equally well known that a taller column of the same liquid is supported by the surface force, when the capillary is *not* in contact with a body of the liquid, Fig. 79b. The additional column of liquid of height  $h_2$  is borne by the surface force originating at the meniscus in the tip of the capillary, and the height of this additional column of liquid is the greater, the smaller the diameter of the opening of the tip. If now the capillary  $b$  is lowered into the surface of the body of liquid, the meniscus in the tip of the capillary is eliminated, and the liquid flows out of the capillary until the upper meniscus has dropped from level II to level I. Of course, the flow may be interrupted at any time by raising the

capillary so that the meniscus in the tip is restored. If the capillary is calibrated as a buret, the meniscus in the tip can take the place of an automatically operating stopcock as long as the meniscus in the calibrated part is on the descent from level II to level I. The phenomenon may be easily observed with the use of a capillary pipet and a few drops of water on a watch glass.

Of course, the distance between level I and level II is only a few centimeters if the diameter of the opening of the tip is of the order of 0.1 mm. Accordingly, the calibrated part of measuring burets employed in a vertical position would have to be made quite short if the operation of the buret is to rely solely on the meniscus in the tip, and the tip cannot be made extremely fine because of the prohibitive amount of time which would be required for the outflow. This difficulty may be overcome in various simple ways:

(a) The height  $h_2$  is easily able to accommodate a bulb of 4-ml capacity, and the standard solution may be weighed instead of measured. The weighing buret of Struszynski<sup>16</sup> is designed according to this principle.

(b) The calibrated capillary is mounted in an inclined or horizontal position. No liquid will leave the tip of the capillary *c*, Fig. 79, as long as it is not touched to the bulk of the liquid below. Contact, however, will empty the horizontal arm completely, no matter how long it is. The automatic microburet with horizontal scale of Anna-Greta Hybbinette, which is described in Expt. 71, belongs to this type.

(c) Burets with long perpendicular scale may be provided with some device for the regulation of the gas pressure above the standard solution in the calibrated tube. The gas pressure is adjusted to counterbalance the weight of standard solution in excess over that carried by the surface force. By regulation of the gas pressure, level I of Fig. 79 is continuously held at such a distance below the meniscus in the calibrated tube that the outflow can be started or stopped by making or breaking the contact of the tip with the surface of the titrated solution.

This last principle has been repeatedly employed in a more or less incidental manner when working with certain microburets which have been derived from the syringe type<sup>17, 18, 19</sup> by inserting an air

<sup>16</sup> M. Struszynski, *Przemysl Chem.*, **20**, 53 (1936).

<sup>17</sup> S. Wermuth, Thesis, Universität Graz, Germany, 1914; F. Emich, *Umsetzungen sehr kleiner Stoffmengen*, in A. Stähler, E. Tiede, and F. Richter, *Handbuch der Arbeitsmethoden in der anorganischen Chemie*, Berlin, W. de Gruyter, 1925, Vol. 2, p. 655.

<sup>18</sup> P. Brandt-Rehberg, *Biochem. J.*, **19**, 270 (1925).

<sup>19</sup> E. M. P. Widmark and S. L. Örskov, *Biochem. Z.*, **201**, 15 (1928).

space between the standard solution and the mercury on which the plunger acts. The air space was inserted to permit the use of standard solutions which otherwise would react with the mercury. The best-known burets of this kind are those of Linderstrøm-Lang and Holter<sup>20</sup> and of Paul L. Kirk,<sup>21</sup> which have been used in the titration of extremely small quantities of material.<sup>22</sup> More obvious applications of the third principle are found in the burets of Schwarz<sup>23</sup> and especially of Heatley.<sup>24, 25</sup> These burets too were designed for work with very small volumes of standard solutions. Heatley uses a mercury leveling bulb to regulate the pressure in the air space. The buret of Schwarz is operated by blowing with the mouth.

Burets purposely based on principle (c) have been designed for the titration of centigram and milligram samples on the one hand and the titration of gamma samples on the other. The burets proper always consist of a capillary with a fine tip, which has some reference mark for the alignment of the millimeter scale which is never etched on the glass tube itself. The wide end of the capillary is flexibly connected to a device for the regulation of the gas pressure above the standard solution. The burets are usually held in a fixed position, and the titrated solutions are moved by means of mechanical devices so as to make or break the contact with the tips of the burets. There is no need for touching such burets during titration, for they are operated by remote control. Burets of this type may be easily prepared in any desired size, used in any position required, and built into apparatus in a manner which excludes direct access to the burets proper.

**Regulation of Pressure for Burets with Remote Control.** The reservoir *R*, Fig. 80, is made as large as practicable so that heating or cooling of parts of the connecting tubes has little influence on the total pressure of the system. If there are considerable temperature fluctuations in the room, the reservoir may be immersed in a large container filled with water. All tubing should be approximately 4 mm in outer diameter. A centimeter scale of 1-m length is placed behind

<sup>20</sup> K. Linderstrøm-Lang and H. Holter, *Compt. rend. trav. lab. Carlsberg*, **19**, No. 14 (1933).

<sup>21</sup> P. L. Kirk, *Mikrochemie*, **14**, 1 (1933).

<sup>22</sup> A review of the work of Linderstrøm-Lang and Holter is easily accessible: D. Glick, *J. Chem. Education*, **16**, 68 (1939). Most of the papers on drop analysis by Kirk and his coworkers have been published in the journal *Mikrochemie* (1933 to 1940).

<sup>23</sup> K. Schwarz, *Mikrochemie*, **13**, 1 (1933); **18**, 309 (1935).

<sup>24</sup> N. G. Heatley, *Biochem. J.*, **29**, 626 (1935).

<sup>25</sup> N. G. Heatley, *Mikrochemie*, **26**, 147 (1939).

the manometer tube  $m$  so that the zero mark is at the level of the water in the reservoir  $R$ . The arrangement of the connecting tubes is shown in  $A$ , Fig. 80. The three-way stopcocks  $a$  and  $b$  and the connecting glass tubes are mounted on a board which is rigidly attached to the table top. This makes it possible to operate the stopcocks with

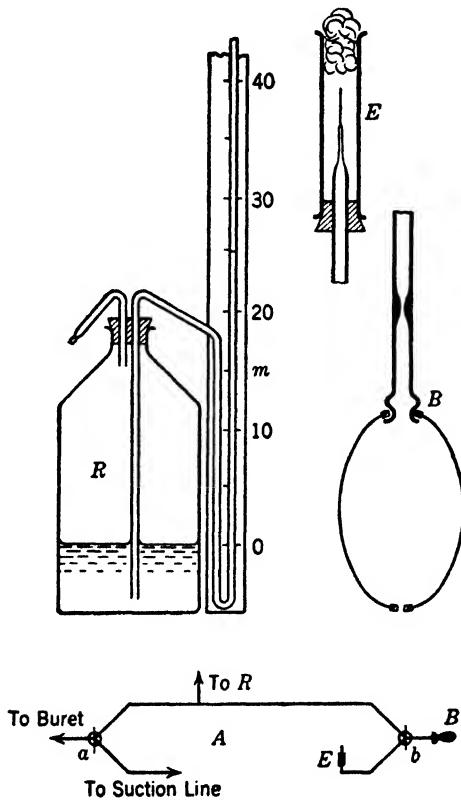


FIG. 80. Apparatus for the regulation of gas pressure.

one hand. The connections to the reservoir and the buret are made with rubber tubing of 2-mm bore. The pressure is supplied by bellows consisting of a small rubber bulb as sold with atomizers for perfume. The bulb is mounted on a short piece of glass tubing, Fig. 80B, which is provided with a capillary constriction so that the pressure is supplied slowly and evenly. For the gradual release of pressure, a capillary exit  $E$  is provided. The fine capillary of approximately 0.1-mm bore is protected by a wide tube into which a plug of cotton is inserted as protection against dust.

**U-Shaped Buret with Remote Control for Centigram and Milligram Procedures.<sup>26</sup>** The buret may be made of thermometer capillary of round uniform bore 0.2 to 2 mm in diameter. At first small bulbs are blown at *d* and *e*, Fig. 81. The short glass tubing with stopcock *c* is fused to the thermometer capillary which is then bent into the form shown in Fig. 81. Finally, the tip of the buret is drawn in two stages. To begin with, the glass around bulb *e* is heated and drawn out so that the bulb is converted to a rather wide capillary, the remainder of which is indicated in Fig. 81. The wide capillary is then drawn out to a fine capillary of 0.02- to 0.2-mm bore, depending upon the capacity of the buret and the rate of outflow desired. A ring mark is etched with hydrofluoric acid below bulb *d*, and then the buret is mounted vertically on a rigid stand. The part of the tube below bulb *d* is finally backed up with a millimeter scale so that the ring mark appears somewhere below the scale division zero. The millimeter scale may be printed on bristolboard or on a strip of any suitable plastic material.

The calibration of the buret, Expt. 72, may be performed before or after the drawing of the capillary tip. After calibration, the buret is cleaned by connecting *c* to the suction device shown in Fig. 14B, inserting the tip in the liquids, and sucking cleaning solution and distilled water through the buret. For drying, air which has been filtered through a plug of cotton is drawn through the buret.

After the capillary has been cleaned and dried, a rubber bulb is connected to tube *c* of the buret. Some paraffin is heated in a test tube until heavy fumes are given off, and then the tip of the buret is dipped into the molten paraffin and slowly withdrawn while all the time a strong pressure is exerted on the rubber bulb so that a continuous stream of air bubbles emerges from the tip of the buret. The pressure on the rubber bulb is released when the paraffin has solidified to form a thin film on the outside of the buret tip.

The buret is now connected at *c* by means of a flexible rubber tubing of 4-mm outer diameter to three-way stopcock *a*, Fig. 80A. Some standard solution is transferred to a dry centrifuge cone and briefly whirled to collect any floating particles in the point of the cone, which is then attached by means of a suitable clamp to the vertical rod of an elevating stand. This vertical rod moves up and down by means of rack and pinion. After preliminary adjustment of the relative positions of buret and elevating stand, the centrifuge cone

<sup>26</sup> A. J. Llacer and J. A. Sozzi, unpublished investigation, Queens College, Flushing, N. Y.

is raised so that the tip of the buret is inserted in the standard solution. The buret is connected through stopcock *a* to the suction line, and the standard solution is drawn into the buret with *slight* suction

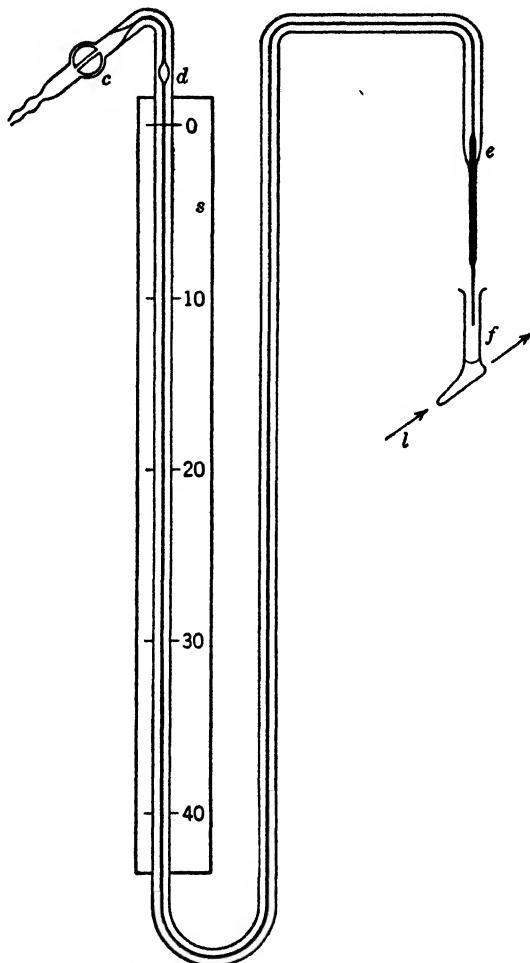


FIG. 81. U-shaped buret with remote control.

to avoid the formation of air bubbles. The solution is allowed to reach the bulb *d*, and then the suction is broken by admitting air through stopcock *T*, Fig. 12, of the trap in the suction line. Stopcock *a* is closed, and, as a rule, the standard solution recedes automatically from bulb *d* of the buret. The meniscus is observed with a magnifying glass, and when it reaches the zero point of the scale, the centrifuge cone is quickly lowered to stop the outflow. (If the meniscus

does not drop to zero by itself, pressure must be applied as described below.)

The next task is the determination of the pressure required to get the critical level II, Fig. 79, slightly below the zero point of the scale. This critical pressure will cause the standard solution to be expelled from the buret even though the tip of the buret does not touch a body of liquid. The critical pressure must be known, for it must never be reached or exceeded when using the buret in titrations. The determination is simple: To begin with, the reservoir *R*, Fig. 80A, is connected through *a* and the suction line to the atmosphere. Then the buret is connected through *a* and *b* to the reservoir and the rubber bellows *B*. By operating the bellows one increases the pressure gradually while observing the meniscus in the buret with the aid of the magnifying glass. When the meniscus begins to move, *b* is closed and the manometer is read. For a control, the pressure is very gradually lowered by connecting through *b* to *E*, and the pressure at which the outflow stops is determined. Finally, standard solution is taken into the buret to bring the meniscus back to just below bulb *d*.

The "operating pressure" is then to be determined for the positions 0, 50, 100, 150, 200, 250, 300, and 350 of the meniscus on the millimeter scale. The operating pressure is defined as the manometer reading which produces a rate of outflow corresponding to a travel of the meniscus of 10 to 20 mm per minute. Of course, the operating pressure must be smaller than the critical pressure at the same point, for otherwise the outflow could not be stopped by withdrawing the body of solution from the tip of the buret. The determination of the operating pressure requires several trials. The meniscus is sucked up into bulb *d*, after which the suction is broken and stopcock *a*, Fig. 80A, is closed. The cone with the standard solution is lowered, and the pressure in the reservoir *R* is adjusted to some likely value. Then *b* is closed, and the buret is connected through *a* with the reservoir. The tip of the buret is immersed in the standard solution, and the rate of travel of the meniscus is timed at the marks 0 and 20 by means of a stopwatch. The timing is repeated at 50 to 70, 100 to 120, etc., until the rate of travel becomes too slow. The experiment is repeated with the necessary changes until all operating pressures have been determined. These important data are collected in tabular form so that they are readily available when the buret is used. They will hold for most acid and neutral standard solutions. Unpredictable variations in the surface energy of alkaline solutions are obviously caused by traces of soap formed on contact with contaminated surfaces.<sup>27</sup>

<sup>27</sup> This explanation has been kindly suggested by Dr. Jerome Alexander.

The use of the buret in titration is quite simple. The meniscus of the standard solution is allowed to drop to the zero mark or just below it. Then the standard solution is removed, and the solution to be titrated is brought below the tip of the buret so that it can be raised or lowered by means of the elevating stand. The connection between reservoir and buret is broken by the three-way stopcock, and then the pressure in the reservoir is built up to the required operating pressure. The buret is connected to the reservoir, and the titration is started by raising the body of solution. When mark 50 has been reached by the meniscus, the titration is interrupted for readjustment of the pressure, etc. As may have been noticed, stopcock *c*, Fig. 81, remains open continuously. In fact, it is not an essential part of the buret, but a desirable safeguard. Whenever a titration must be interrupted for a short time (back titration with standard solution from another buret), stopcock *c* is closed to assure constancy of the gas pressure above the standard solution.

When not in use, microburets of this type are emptied, rinsed with water, and dried. Evaporation of the standard solution at the opening may otherwise cause clogging of the tip. When standing for a short time, the tip of the buret is best immersed in some standard solution held ready for this purpose in a suitable tube. It may be added that the zero reading should be taken immediately after removing the tip of the buret from this solution. A displacement of the meniscus in the calibrated part of the buret may be observed when the titration is started after some delay. Nevertheless, the original zero reading must be retained, since the evaporation takes place at the tip, resulting essentially in the loss of solvent and formation of a more concentrated solution in the tip. Since this concentrated solution is expelled at the start of the titration, the amount of active agent indicated by the displacement of the meniscus is transferred to the titrated solution, and the use of the original zero reading is correct.<sup>28</sup>

It cannot be denied that considerable practice is needed for the efficient use of the buret with vertical scale. The effort is justified, however, since this type of buret permits an extraordinarily fine control of the flow of standard solution.

**Buret with Remote Control for the Titration of Gamma Samples.**<sup>29</sup> Table V, which assumes that millimeter rulings are used for the graduations of microburets, gives very small diameters for the bore of the calibrated capillaries. A 40-mm capillary of 0.035-mm

<sup>28</sup> S. Siggia, manuscript in preparation, Queens College, Flushing, N. Y.

<sup>29</sup> Anne G. Loscalzo, preliminary reports, Washington Square College, New York University.

uniform bore could be made and operated as buret. It seemed preferable, however, to give the capillary a bore of 0.2 mm and to reduce the length of the graduated portion to 2 mm. This can be done by observing the position of the meniscus with the aid of a low-power microscope equipped with an eyepiece micrometer. The objective is selected so that 100 or 200 divisions of the micrometer scale correspond to a distance of approximately 2 mm on the object.

A capillary of approximately 0.2-mm bore is drawn out at one end of a capillary of 0.5- to 1-mm outer diameter, which fits into the pipet holder of a manipulator, Figs. 45 and 46. The copper tubing of the pipet holder may be used to make the connection to the pressure reservoir, Fig. 80. The bore of the fine capillary will be found sufficiently uniform over a distance of 2 mm, and a reference mark is easily obtained by placing a fiber, spun from thick Canada balsam, halfway around the outside of the thin capillary. The tip of the buret is drawn out by means of the technique employed in preparing micropipets. The diameter of the opening is a few microns.

The buret may be used in any position desired, and the critical pressure, which causes outflow of standard solution while the tip of the buret is in air, is of the order of 1.1 atmospheres.

### TITRATION VESSEL

Paradoxical as it may appear at first, the design of a suitable container for the solution to be titrated offers more difficulties than that of the buret. The reason for this is immediately apparent when one reviews Table V and recalls that the volume of the solution to be titrated should be approximately half of the useful capacity of the buret:  $0.5c$ . This means a volume of 0.02 ml for the titration of milligram samples with standard solutions of the same strength as used in macroanalysis, and it prescribes a volume of 0.02 ml for the titration of gamma samples. Up to the present time one hundred to several thousand times larger volumes have been titrated with ten to one hundred times more dilute standard solutions than customarily employed in macroprocedures,<sup>1, 18, 20, 21</sup> and, in addition, higher concentrations of indicator than in macroanalysis were frequently employed. The consequences, lack of sharpness in the end points and the necessity of applying indicator corrections,<sup>1, 10</sup> were accepted, since the titration of the above-mentioned small volumes of solutions appeared still less practical.

Let us assume that 1 gram of sodium carbonate has been dissolved in 20 ml of water. The solution is treated with 3 drops (0.1 ml) of

indicator solution and is ready for titration with 0.5 *N* hydrochloric acid from a 50-ml buret. Keeping all concentrations unchanged, one properly shifts to the milligram scale by taking one-thousanth of the carbonate solution (0.02 ml) and employing a buret of one thousand times smaller capacity. If, however, 0.02 ml of the above carbonate solution is transferred to a correspondingly small flask, the color of the indicator is no longer recognizable, for the thickness of the layer of solution, which was several centimeters in the Erlenmeyer flask, is now only a few millimeters. If, on the other hand, a capillary of 0.8-mm bore is used for a titration vessel, the solution will fill a 40-mm length of it, and the color of the indicator will be visible. Such a titration vessel, however, will make it troublesome to mix the solution during the titration, and there are other obvious difficulties. A perfectly satisfactory solution of the problem is hard to find, since some of the requirements are virtually opposed to one another: the volume of the solution should be small but a thick layer of it should be available, and a tube containing the solution should be long and narrow for the observation of color but sufficiently wide for stirring and the admission of the buret tip.

Nevertheless, several approaches are open as far as the titration of centigram and milligram samples is concerned. The boot-shaped titration cone *f* of Fig. 81 has been much used by Llacer and Sozzi.<sup>26</sup> Light was sent through the solution in the direction indicated by arrow *l*, and the color change of methyl red could be nicely observed if a brightly illuminated white surface was placed at *l*. Stirring was performed by bubbling air through the solution by means of a fine capillary, Fig. 40. Air of the desired pressure is best obtained by means of two aspirator bottles which may have a capacity of only a few hundred milliliters, since the volume of air required is surprisingly small. The air is freed from carbon dioxide by passing it through a tube with Ascarite, the outlet of which should be provided with a stopcock so that the stream of air may be stopped at any time. A screw clamp for the regulation of the rate of flow is placed on the flexible rubber tubing connecting the Ascarite tube to the tube ending in the fine capillary. The latter tube is held by a clamp attached to the vertical rod of the elevating stand which supports the boot-shaped cone. The capillary must be inserted so that its opening is in the point of the cone. Obviously, it is inconvenient that both the capillary and the tip of the buret must be admitted through the same opening of the cone.

Another solution is shown by Fig. 82. The titration vessel consists of the titration flask *t* and the coloroscopic capillary *c* (compare Expt.

46). To facilitate the observation of color the apparatus is best made of a material (glass, clear fused quartz, or plastic) which is colorless or nearly colorless. The dimensions are listed in Table VI as functions of the useful capacity  $c$  of the microburet selected. The bulb of the titration flask is always given a capacity somewhat larger than  $2c$ ,

TABLE VI  
DIMENSIONS OF TITRATION FLASKS AND  
COLORISCOPIC CAPILLARIES

$c$ in $\lambda$	Bulb		Capillary	
	Diameter in mm	Capacity in $\lambda$	Bore in mm	Capacity in $\lambda$
40	6	110	0.6	11
400	12	860	1	30

and the capacity of the coloroscopic capillary is held below  $0.5c$  while the length is maintained equal to 40 mm. The tip of the buret is admitted through the neck of the flask  $t$ . The coloroscopic capillary may be used for passing an inert gas through the solution. The gas is supplied through the opening  $a$  of the T-tube  $h$ , the other two openings of which are sealed. At  $r$  the seal is formed by a piece of rubber tubing. At  $o$  the tube is fused shut so that a thin, plane membrane of glass is obtained which serves as a window for the observation of the liquid in the capillary. A beam of light is sent to the bulb of the titration flask as indicated by arrow  $l$ , and the eye of the observer is protected by a circular opaque shield  $s$ . The clamp of the elevating stand is applied to leg  $h$  of the T-tube, which is then connected by means of flexible rubber tubing to a 120-degree three-way stopcock which permits connecting the T-tube either to the atmosphere or to the supply of inert gas. A screw clamp between the three-way stopcock and the train for the purification of the gas serves for the regulation of the rate of flow.

The coloration in the capillary cannot be observed at the same time that stirring is performed. The intermittent operation is not objectionable, however, for observation of the indicator color serves no purpose as long as the titrated solution has not become homogeneous by stirring. When finally the T-tube is connected to the atmosphere, the liquid in the titration flask is drawn into the coloroscopic capillary

by the surface energy. If the coloroscopic capillary is properly inclined to the horizontal, it will fill up to its evenly cut end at *o*, and the color of the liquid can be easily observed with or without the aid of a magnifying glass. The width of the opening of the fine tip of the coloroscopic capillary which is sealed into the titration flask is the result of a compromise. The diameter of the opening must be 0.1 mm or more so that the capillary fills and empties with reasonable speed.

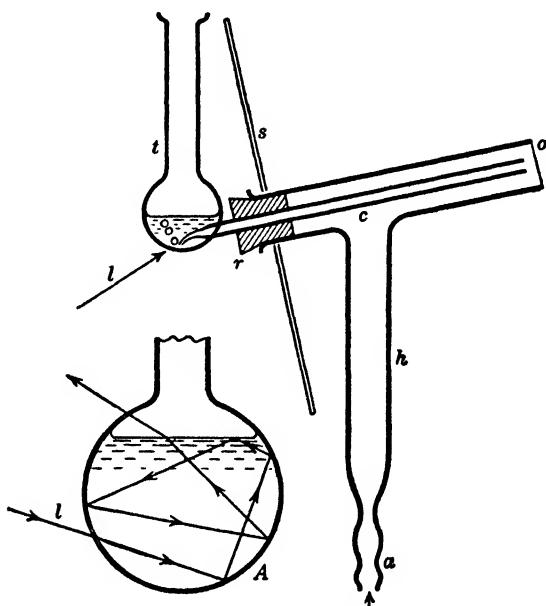


FIG. 82. Titration vessels.

The relatively large opening makes it necessary to regulate the flow of inert gas (air) so that splashing of the titrated solution is avoided. Before the final adjustment of the end point the walls of the titration flask may be rinsed either by means of an atomizer or by boiling the titrated solution so that the ring of condensate is for a short time driven up near to the opening of the flask.

Siggia<sup>28</sup> prefers to introduce the gas used for stirring the titrated solution through a fine capillary, Fig. 40, which is inserted through the wide neck of the titration flask *t*, Fig. 82. Opening *a* of the T-tube is provided with a long rubber tubing fitted with a mouthpiece at the other end. The solution which enters the coloroscopic capillary *c* is held there for the final adjustment of the end point. Standard solution is run into the major portion of the solution in the bulb with the maximum permissible speed until the end point is overstepped. Even

with the proper concentration of indicator it is possible to recognize roughly the color change of the liquid in the bulb. Now the contents of the coloroscopic capillary are transferred to the bulb by cautious blowing with the mouth, and the titration is finished by adding small volumes of standard solution at a time and employing the coloroscopic capillary for the observation of color after each addition of standard solution. This procedure offers several advantages. The bubbles of inert gas may be made any desired size, since the gas is not introduced through the coloroscopic capillary. Back titration with the contents of the coloroscopic capillary permits rapid approach of the end point, and the use of a second buret with a standard solution for back titration may be dispensed with.

The titration of *gamma samples* with standard solutions of the customary concentrations may be performed in capillary cones (Expts. 57, 58). The stirring may be done with the tip of the buret, but it will be necessary to employ a higher concentration of indicator than usual, provided that no way can be found to make the light repeatedly pass through the titrated solution as suggested by Fig. 82A.

#### INDICATION OF THE END POINT

The discussion in the preceding section applies to all titrations in which the end point is recognized by a change in the color of the titrated solution. It applies to neutralimetry with the use of dye indicators, redox titrations employing redox indicators, and titrations with permanganate, iodine, or thiocyanate, i.e., to all methods producing a color change throughout the titrated solution. If the color change is localized on particles of a precipitate<sup>28</sup> (Mohr's method, use of adsorption indicators) or in a tiny drop of a liquid which does not dissolve in the titrated solution<sup>28</sup> (coloration produced by iodine in a droplet of chloroform), the recognition of the color change is considerably facilitated, and the restrictions emphasized in the preceding sections do not apply.

Solutions of indicators should be used with the same strength as in macrotitrations, and the volume of indicator solution used in each titration should be reduced in the same proportion as the amount of sample. If the useful capacity of the macroburet is assumed to be  $c = 40$  ml (and 3 drops [approximately 0.1 ml] of indicator solution is taken), the volume of indicator solution may be expressed as  $0.0025c$ . Again referring back to Table V, it may be seen that the volumes of indicator solution will be of the order of  $1 \lambda$ ,  $0.1 \lambda$ , and  $0.1 m\lambda$ , depending upon whether centigrams, milligrams, or gammas

of material are taken for a sample. Volumes of the first two orders may be easily measured with the aid of capillaries of 0.4-mm and 0.2-mm bore, respectively. If it is permissible to do so, it will be found more convenient to incorporate a suitable amount of indicator into the standard solutions. (In the above example,  $0.0025c$  volumes of indicator solution would be added to  $c$  volumes of standard solution.)

The use of electrometric end-point indication in microtitrimetry does away with the problem of the observation of color. It has the additional advantage that the use of very dilute solutions and standard solutions is permissible in conductometric titrations and in redox titrations using potentiometric end-point indication. Suitable titration cells have been described for centigram and milligram procedures<sup>1, 30</sup> and for gamma procedures.<sup>11, 22, 31, 32</sup> The amount of current taken by the potentiometer may have to be considered, since 1 milliampere-second causes reaction of 0.01  $\gamma$ -val of an ion (e.g., may deposit 1  $\gamma$  of silver).

### STANDARD SOLUTIONS

It has been pointed out repeatedly that it is desirable to use standard solutions of the same normality as in macroanalysis. If the stability of the indicator permits, it is often convenient to incorporate it into the standard solution. The concentration of the indicator in the standard solution is made identical to that in the titrated solutions of the macrotitrations. Special attention must be given to the absence of solid particles in the standard solutions, which are frequently responsible for the clogging of the tips of microburets. The problem of storing standard solutions is discussed at length by Lindner<sup>33</sup> and Mika.<sup>1</sup>

### PRACTICE EXPERIMENTS IN MICROTITRIMETRY

The following directions apply to determinations on centigram samples and the necessary preliminary work. Centigram procedures have been selected for the experimental work for several reasons. The apparatus is simple, inexpensive, and may be easily prepared by the

<sup>30</sup> A. A. Benedetti-Pichler, *Z. anal. Chem.*, **73**, 200 (1928).

<sup>31</sup> K. Linderstrøm-Lang, A. H. Palmer, and H. Holter, *Z. physiol. Chem.*, **231**, 226 (1935).

<sup>32</sup> K. Schwarz, *Mikrochemie*, **13**, 6 (1933).

<sup>33</sup> J. Lindner, *Mikrochemie, Molisch-Festschrift*, p. 301 (1936).

experimenter himself. The preparation of the required apparatus is a valuable aid to thorough understanding, and the necessary changes which have to be made for the titration of milligram samples should appear obvious from the preceding general discussion of principles. Finally, the need for a microchemical balance or a very precise analytical balance is definitely eliminated.

It may be pointed out that the microburets used in the practice experiments may be employed for a rather accurate estimation of the quantities of constituents isolated in the course of a qualitative analysis. The procedure of analysis proposed by Swift<sup>34</sup> may thus be extended to centigram and milligram samples.

### EXPERIMENT 71

#### Preparation of Two Automatic Microburets with Horizontal Scale<sup>35</sup>

##### *Required:*

Wooden boards; saw; drill; screws; screwdriver; 2 nuts; 2 bolts; 8 washers; fine copper wire; thumbtacks; some nails.

2 scales, 0 to 20 cm, on bristolboard.

2 thermometer capillaries, approximately 2-mm uniform bore, Pyrex, 40 cm long.

Pyrex tubing, 12-mm. outer diameter, 20 to 30 cm. long.

Hydrofluoric acid; beeswax.

Platinum crucible, Bakelite beaker, or glass beaker coated with wax.

Suction device, Fig. 14B.

The construction of simple wooden stands for the burets is shown in Fig. 83A and does not need further explanation. The nut *b* is tightened so that the horizontal arm does not move too easily. Two washers should be placed between the boards at *b*. A series of small holes are drilled through the vertical board at *e* so that nails placed in these holes may serve as stops to limit the motion of the horizontal board.

The capillary which is to form the buret proper is bent as indicated in the figure. Then the tip is drawn out in two steps, first to a capillary of approximately 2-mm outer diameter and then to a gradually tapering fine capillary. The proper form of the tip must be found by trial. The length *a* of the perpendicular part of the tube and the tip are adjusted so that, with the long part of the tube in a horizontal position, water will flow out only when the tip touches a

<sup>34</sup> E. H. Swift, *A System of Chemical Analysis for the Common Elements*, New York, Prentice-Hall, 1939.

<sup>35</sup> Anna-Greta Hybbinette and A. A. Benedetti-Pichler, *Mikrochemie* (1941).

wet surface. At the same time, the rate of flow must be of such an order that the meniscus in the horizontal part of the tube travels approximately 15 mm per minute. These conditions are fulfilled if *a* is given a length of 3 to 4 cm and the diameter of the opening of the tip is made approximately 0.1 mm. Leaving the gradually tapering, fine capillary of the tip quite long to begin with, the buret is filled with water and tested. It is sufficient to place the middle portion of the

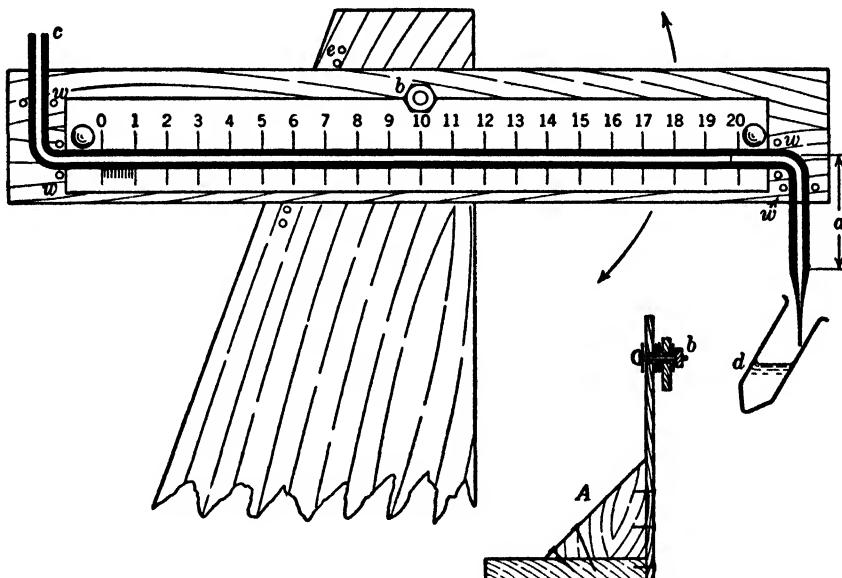


FIG. 83. Automatic microburet with horizontal scale.

tube on a millimeter rule and to hold both with the hand while making the tests. Short pieces of the tip are cut off until operation of buret and rate of flow comply with the above given specifications. If the buret is intended for exclusive use with a certain standard solution, it is advisable to perform the adjustment of the tip with that standard solution. This will better assure that the desired rate of flow is attained in actual use with the graduated capillary in horizontal position.

A ring mark is etched near the tip end of the calibrated portion of the thermometer capillary so that the mark will be situated between the nineteenth and twentieth centimeter of the scale when the buret is mounted. The millimeter scale is fastened to the horizontal board of the stand by means of thumbtacks. The burets are attached to the boards by means of copper wire which is drawn through the holes *w*, Fig. 83.

The wide Pyrex tubing is used to prepare one or two pairs of centrifuge cones of 2-ml capacity and the form shown in Fig. 83d. They fit the large holes of the wooden block, Fig. 6. A few stirring rods, Fig. 4D, are made, and the stirring rods and cones are immersed in cleaning solution and kept there until they are needed.

Figure 83 shows a buret in the working position. Rotation of the horizontal arm of the stand through 180 degrees brings the buret into the cleaning position. Then the opening *c* is connected by means of the device, Fig. 14B, to a suction flask. After the suction is started, cleaning solution and wash liquids are applied dropwise to the tip with the aid of a glass rod. The buret is finally dried by sucking air through it, or it is rinsed with the standard solution to be used. If the latter course is taken, it is important to do the final rinsing with very slight suction to avoid evaporation.

## EXPERIMENT 72

### Calibration of Microburets

Mercury, 1 ml, clean, dried by passing it through filter paper.

Rubber tubing, glass rod, screw clamp.

The total capacity of the buret, described in the preceding experiment, is approximately 0.6 ml. Each millimeter of the scale corresponds to approximately  $3 \lambda$ , and if the positions of the meniscus are read with a precision of  $\pm 0.1$  mm, the determination of volume will be reliable within approximately  $\pm 0.4 \lambda$ .

If desired, the total delivery of the burets may be determined by weighing the water which is expelled when the meniscus travels from zero to the 200-mm mark. The directions have been outlined in the section on pipets. The rate of outflow must be the same as when the buret is used for titration. Experience has shown that practically no drainage error is introduced if the meniscus travels as slowly as 10 to 20 mm per minute. The slowly moving meniscus seems to wipe off the walls of the buret so that only a thin film of liquid is left behind. It seems plausible that the efficiency of the wiping action may be determined by the bore of the tube.

The uniformity of the bore of the graduated part of the buret must be tested. This is done by measuring the length of a thread of mercury in the various parts of the capillary.

Both burets are carefully cleaned and dried. The calibration for uniformity of bore is performed as follows.

The buret is inclined so that the tip end is slightly raised. Then small drops of mercury are introduced through the wide opening *c*, Fig. 83, by means of a capillary pipet until a thread close 10 mm long has formed. A rubber tubing approximately 10 cm long is attached

to *c*, and the open end of this tubing is closed by inserting one end of a glass rod of the same length as the tubing. Now, the nut *b* is removed, and the board with the millimeter scale and the buret is placed on the table top. The thread of mercury is brought to any desired position by moving the glass rod in the rubber tubing. The fine adjustments are made by means of a screw clamp which is placed on the rubber tubing.<sup>36</sup> Warming of the buret with the hands must be avoided, and, of course, it is desirable to work in a room in which no sudden changes of temperature occur. The aid of a magnifying glass is desirable for reading the positions of the menisci.

The mercury meniscus facing the end *c* of the buret is brought to the positions 0, 1, 2, 3, 4, 5, ... to 19 (cm), and the positions of the other meniscus are recorded. Then the meniscus facing the tip end of the buret is brought into the positions 20, 19, 18, 17, ... to 1 (cm), and the positions of the other meniscus are recorded. These two series of readings are taken to counteract the effects of a gradual rise or drop of the temperature. In addition, a control is provided which excludes the possibility of committing grave mistakes.

An example of record and calculations is shown in Table VII. In column 4 the volume of each interval is expressed as a function of the unknown volume *v* of the thread of mercury. If the thread is exactly 10.0 mm long, the volume of the interval is *v*; if the thread is longer or shorter than 10.0 mm (e.g., 10.25 mm), a correction has to be added to *v* (e.g., *v* — 0.25 MM). Of course, the correction too has the dimension volume, and the arbitrary unit MM is used as unit of volume. The unit MM represents the volume a 1-mm length of a capillary of *uniform* bore, a 200 mm-length of which has the same capacity as the real capillary between the marks 0 and 20 (cm). It is obvious that the corrections themselves should be corrected because of the variations of the bore. This may be neglected, however, if the corrections are small, i.e., if one has taken care to make the length of the thread very close to 10 mm.

Addition of the items of column 4 gives the capacity of the graduated capillary between the marks 0 and 20 as a function of the unknown volume *v* of the mercury drop and the algebraic sum of the corrections. This volume is set equal to 200 MM, and the equation is solved for *v*.

Addition of the values given in column 4 leads to the figures listed in column 6, which represent the capacities for the first, the first two, the first three, etc., centimeters of the scale. The figures of column 7

<sup>36</sup> A syringe device has been described by Lillias D. Francis, *J. Lab. Clin. Med.*, 22, 718 (1937).

are obtained by substituting the calculated volume of  $v$  into the corresponding items of column 6.

TABLE VII  
CALIBRATION OF GRADUATED CAPILLARY

Interval	Length of thread of mercury		Volume of intervals MM	Interval from zero to	Volume	
	Observed mm	Mean mm			MM	MM
1	2	3	4	5	6	7
0-1	10.3					
1-0	10.2	10.25	$v - 0.25$	1	$v - 0.25$	9.7
1-2	10.3					
2-1	10.3	10.3	$v - 0.3$	2	$2v - 0.55$	19.4
2-3	10.1					
3-2	10.2	10.15	$v - 0.15$	3	$3v - 0.70$	29.2
.	.		.	.	.	.
.	.		.	.	.	.
.	.		.	.	.	.
19-20	9.7					
20-19	9.8	9.75	$v + 0.25$	20	$20v + 0.60$	200.0
Total from 0 to 20:			$20v + 0.60$			

$$\text{Since } 20v + 0.60 = 200 \text{ MM}$$

$$v = 9.97 \text{ MM}$$

The values of column 7 may be used to plot a calibration curve on millimeter graph paper. It is understood that the arbitrary unit of volume MM is a function of the total capacity of the buret. The value of 1 MM changes from buret to buret.

The results of the calibration may be easily given in absolute units of volume. Of course, the volume of water which is delivered when the buret is drained from mark 0 to mark 20 must be determined. Assuming that it has been found equal to  $C$ , one need only multiply each item of column 7 by  $C/200$  in order to obtain the results of the calibration in absolute terms. It must be kept in mind, however, that a capillary, which delivers 576.8  $\lambda$  of water at 20° C may not deliver this same amount of standard solution even if temperature and rate of outflow are kept constant.

## EXPERIMENT 73

Determination of Acid-Base Ratio, Precision of Burets<sup>85</sup>

0.5 *N* hydrochloric acid, clear.

0.5 *N* sodium hydroxide, "free" from carbonate, clear.

Methyl red.

The standard solutions are prepared as usual and poured into 250-ml bottles with rubber stoppers. Their normality need not be carefully adjusted, and it is not necessary to standardize them. Methyl red indicator solution is added to the acid and the base so that they appear just distinctly colored in the bottles.

The burets are cleaned, and rinsed with water and finally with standard solution. Only very light suction must be employed when rinsing with standard solution, and the suction is broken immediately after the last rinse. The buret is returned to the working position and filled with the standard solution, which now may be taken directly from the bottle. The tip is inserted in the solution, and *c*, Fig. 83, is connected to the suction line. Slight suction not exceeding 0.1 atm. should be employed.

As an alternative, standard solutions may be kept in and supplied from U-tubes, Fig. 84. The tip of the buret is inserted through *a*, and pressure is supplied through *b* by means of rubber bellows. After use, *a* and *b* are closed by means of rubber tubings and glass rods. It is obvious that a device of this kind offers definite advantages whenever the standard solution contains a rather volatile constituent.<sup>12</sup>

With each buret the standard solution is taken up just beyond the zero mark of the scale. The tip is withdrawn from the standard solution, and its outside is dried with filter paper. A few drops of standard solution are transferred by means of a pipet to a titrating cone *d*, Fig. 83, for a redetermination of the rate of flow. The graduated capillary is brought into a horizontal position, and the titrating cone is raised by hand so that the tip of the buret touches the solution in the cone. The meniscus in the graduated capillary should move through a distance of 10 to 20 mm per minute. If the rate of flow is outside the indicated range, the buret is slightly tilted, and the test is repeated until the angle of inclination is found which produces the desired rate of delivery. This position of the horizontal arm of the stand is marked with a pencil on the vertical board. If an inclination of the graduated capillary of more than 10 degrees to the horizontal is required, it becomes necessary to prepare a new tip which is ad-

justed to give the desired rate of flow with the graduated capillary in a horizontal position.

Both burets are finally filled again so that the menisci are above the zero marks. The tips of the burets are wiped dry, and then the menisci are brought into the scale and close to zero by touching the tips of the burets to the proper standard solutions. The positions of both menisci are read and recorded.

A clean titrating cone and a stirring rod are carefully rinsed with distilled water. The cone is raised by hand so that the tip of the *properly inclined* sodium hydroxide buret touches the moist wall of the cone, Fig. 83. Approximately 10 to 15 cm of this standard solution is allowed to flow into the cone.

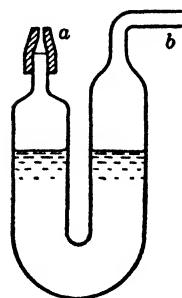


FIG. 84. Container for standard solutions.

Now the titrating cone is best clamped into a stand and raised so that the tip of the acid buret touches the wall of the cone when the graduated part of the capillary is inclined to give the proper rate of delivery. The flow may be stopped at any moment by raising the tip of the buret. (If the titrating cone is held by an elevating stand, the cone is easily moved up and down, and the buret may be continuously left in its proper position.)

The standard acid is added in a continuous stream while stirring with the rod until the titrated solution turns a definitely red color.

Next, standard base is cautiously added until the color changes to brown or yellow. Then acid is added to just restore the red coloration, and then an excess of 1 mm of the acid is added. The contents of the cone are carefully mixed, and any parts of the standard solution which may cling to the walls of the cone are stirred into the bulk of the solution. If, on mixing, the solution should turn yellow, the red color must be restored by adding standard acid, and an excess of 1 mm of the acid. (The same rule holds if the alkaline reaction should return during the heating period which is to follow.) The solution is heated on the steam bath for 2 minutes to eliminate carbon dioxide. It is then cooled to room temperature by running tap water over the outside of the cone, and the titration is finished by adding minute quantities of sodium hydroxide standard solution, stirring after each addition, until the titrated solution acquires a pure yellow coloration. Small portions of standard solutions are added by touching the tip of the buret momentarily to the wall of the cone or the surface of the solution. When the end point is approached, care must be taken to

make certain that liquid adhering to the walls of the titrating cone is stirred into the bulk of solution. If desired, all the liquid may be collected in the point of the cone by brief whirling in the centrifuge.

When the end point has been established, the positions of the menisci in both burets are read and recorded. Then, 1 more centimeter of the standard base is run into the titrated solution, and the end point is again approached and adjusted as outlined above. This is repeated three times.

Although neglect of the following precaution is less serious in centigram procedures, the experimenter is warned not to allow the lapse of too much time between withdrawal of the last portion of standard solution and reading the position of the meniscus. Evaporation of standard solution from the tip of the buret would introduce significant errors in milligram procedures. It might be just as well to make it a common practice in all microtitrations always to read the position of the meniscus immediately after the last portion of a particular standard solution has been withdrawn from the buret.

The burets are cleaned and dried when no longer needed. A sample of the record and calculations is given in Table VIII.

TABLE VIII  
DETERMINATION OF THE ACID-BASE RATIO

NaOH Buret 1, 18 mm/min.		HCl Buret 2, 12 mm/min.		NaOH CM	HCl CM	CM HCl CM NaOH	Devia- tion from mean
Observed cm	Corrected CM	Observed cm	Corrected CM				
0.12	0.12	0.05	0.05				
15.21	15.23	14.86	14.82	15.11	14.77	0.978	+0.003
16.10	16.14	15.71	15.69	16.02	15.64	0.976	+0.001
16.95	17.04	16.50	16.51	16.92	16.46	0.973	-0.002
18.32	18.37	17.74	17.79	18.25	17.74	0.972	-0.003
19.45	19.43	18.82	18.90	19.31	18.85	0.976	+0.001
Arithmetical mean						0.975	±0.002
Acid-base ratio						0.975±0.001 (±1 part per 1000)	

It is obvious that the most trustworthy acid-base ratio is represented by the mean of the five determinations. Since acid and base

have been measured in arbitrary units of volume CM, the acid-base ratio holds for this pair of standard solutions only if they are dispensed from the same burets as used in the determination of the ratio. Of course, it would not be permissible to interchange the acid buret and the base buret, and the numbers of the burets are an essential part of the record.

An average deviation of  $\pm 2$  parts per 1000 for a single determination of the ratio is satisfactory. The average deviation of the mean ( $\pm 0.001$ ) is obtained by dividing the average deviation of the single determination ( $\pm 0.002$ ) by the square root of the number of determinations. With such a small number of determinations as five, it is sufficient to list precision with one significant figure.

For the determination of the titers of the two standard solutions it is sufficient to standardize one of them. Potassium biiodate, sulfamic acid, and ammonium chloride are recommended as standards. Approximately 0.25 mg-val (97 mg, 24 mg, and 13 mg, respectively) are weighed off accurately and titrated. The balance must be sufficiently precise so that the average deviation of a single weighing is equal to or less than 0.0005 times the weight of the standard substance. The use of a precise balance may be altogether avoided if standard solutions of the above substances are prepared on a large scale, and small aliquots are measured off by means of micropipets.

Potassium biiodate and sulfamic acid are simply titrated as strong acids. Ammonium chloride is treated in the titrating cone with a drop of water and with an excess of sodium hydroxide standard solution. The cone is then placed on the steam bath, and its contents are evaporated to dryness while a stream of air is blown into the cone. The air should be freed from carbon dioxide for this purpose. The residue is dissolved in a drop of water and titrated with acid and base as done in the determination of the ratio. The change from red to yellow should always be taken for the end point.

## EXPERIMENT 74

### Determination of the Titer of Thiosulfate Solution<sup>34, 35</sup>

Wire of pure electrolytic copper, weight per centimeter known.

Precise analytical balance, or pipet and standard copper solution.

0.1 N thiosulfate solution. The calculated quantity of thiosulfate is dissolved in water, and 0.4 ml of chloroform is added for each liter of solution.<sup>37, 38</sup>

Potassium iodide, c.p., solid.

Starch, indicator solution.

The surface of the copper wire is carefully cleaned, and, with clean shears, a length of it is cut off which corresponds to approximately

<sup>37</sup> J. L. Kassner and Esther E. Kassner, *Ind. Eng. Chem., Anal. Ed.*, **12**, 655 (1940).

<sup>38</sup> F. J. Kirkish, *Chemist-Analyst* (1940); *C.A.*, **34**, 6895 (1940).

3-mg weight. The wire is caught on a clean sheet of paper and transferred by means of forceps to a small watch glass which has been weighed empty. The wire is weighed on a balance which has a precision of  $\pm 0.015$  mg, or better, for a single weighing.

The wire is then transferred into a titrating cone, which is covered with a small glass bulb, Fig. 75B. The metal is treated with 0.2 ml of 3 *M* nitric acid, and, if necessary, the mixture is heated on the steam bath until the metal is completely dissolved. The bulb is rinsed into the cone with 3 drops of water. The contents of the cone are collected in the point by means of the centrifuge, and then the cone is placed on the steam bath. A stream of air is blown into the cone, and its contents are evaporated to dryness. The residue is dissolved in 3 drops of 6 *M* sulfuric acid, and the solution is again evaporated on the steam bath. The cone is finally transferred to a heating block, Fig. 9, and, while air is blown into the cone, the temperature is quickly raised until heavy white fumes are liberated. The cone is cooled by running tap water over the outside, and then the contents are diluted by adding 0.3 ml of water. Concentrated ammonia is added from a pipet until the intense blue color of the ammonia complex appears. Then 6 *M* acetic acid is added from a measuring pipet until the color of the cupric ion is restored. Finally an excess of 0.03 ml of the acetic acid is added.

The thiosulfate buret is made ready for use as outlined in the preceding experiment, and the zero reading is taken. Then, 20 to 30 mg of potassium iodide (solid) is added to the copper solution, and, while the mixture is stirred with a rod, standard thiosulfate is added until the color of the iodine is nearly discharged. Then 0.05 ml of starch solution is added from a measuring pipet. The titration with thiosulfate is continued to the disappearance of the blue color, and the position of the meniscus in the buret is read.

After the first preliminary trial, the determination of the titer may be repeated with two or three more samples of copper wire. The buret is cleaned and dried when no longer needed. The record is kept in the form of Table VIII. The titer is expressed as the ratio mg copper per CM thiosulfate. Again, the number of the buret and the rate of delivery are essential parts of the record.

## EXPERIMENT 75

Determination of the Titers of Cyanide Solution and Silver Nitrate Solution<sup>35</sup>

Wire of pure silver, weight per centimeter length known.

Precise analytical balance, or pipet and silver solution of known titer.

0.2 *M* standard potassium cyanide. It is prepared by dissolving 13 gram KCN and 5 gram KOH in 1 liter of water.

0.1 *M* standard silver nitrate.

1 *M* potassium iodide.

Liebig's method for the determination of cyanide may be used for the estimation of silver.<sup>34</sup> The silver solution is first treated with an excess of potassium cyanide to convert all silver into the complex ion  $\text{Ag}(\text{CN})_{-2}$ . Then potassium iodide is added, and the solution is titrated with standard silver nitrate until a permanent turbidity of silver iodide is formed:  $2\text{CN}^{-} + \text{Ag}^{+} \rightleftharpoons \text{Ag}(\text{CN})_{-2}$ . Strong lateral illumination and observation against a dark background are essential for the recognition of the end point.

As the titer of the standard cyanide solution changes quite rapidly, it must be redetermined every day. This can easily be done when the titer of the standard silver nitrate is once known.

**DETERMINATION OF THE TITER OF STANDARD CYANIDE.** The surface of the silver wire is carefully cleaned. Three lengths of the wire, each of them corresponding to slightly less than 5 mg of silver, are cut off by means of clean shears and caught on a sheet of paper. The pieces of wire are weighed separately and then transferred into three separate titrating cones which must be clearly marked. The balance should give an average deviation for a single weighing of  $\pm 0.025$  mg or better. Each sample of silver wire is treated as follows.

After the opening of the cone is covered, the silver is treated with 0.2 ml of 3 *M* nitric acid. If necessary, the mixture is heated on the steam bath until the metal is completely dissolved. The bulb is then rinsed into the cone with 3 drops of water, and the contents of the cone are evaporated to dryness. The residue is dissolved in 0.3 ml of water. The solution is treated with 0.05 ml 6 *M* ammonia, and the contents are collected in the tip of the cone by means of the centrifuge.

The burets are filled with standard cyanide and standard silver nitrate, and the zero readings are taken. The solution in the titrating cone is treated with 0.01 ml of 1 *M* potassium iodide. The cone is then inserted into a clamp so that it has the proper position for the titration with standard cyanide. A piece of black paper is placed behind the cone, and light from a microscope lamp is sent from the

side into the cone. Stirring continuously, one adds standard cyanide until the precipitate of silver iodide is dissolved and the titrated solution has become completely clear. The cyanide is added in small portions toward the end, and the mixture is vigorously and thoroughly stirred after each addition so that no considerable excess of the standard solution will be employed. The titration is finished with standard silver nitrate, which is added in very small portions until a faint permanent turbidity is obtained in the titrated solution. The positions of the menisci in both burets are read and recorded.

**DETERMINATION OF POTASSIUM CYANIDE-SILVER NITRATE RATIO.** The determination of the ratio must follow immediately after the titrations for the determination of the titer of the cyanide solution so as to prevent the titer of the standard cyanide from changing in the meantime.

The burets are filled with standard cyanide and standard silver nitrate, and the zero readings are taken. Approximately 15 cm of the standard cyanide is run into a titrating cone. From a measuring pipet are added 0.05 ml 6 *M* ammonia and 0.01 ml 1 *M* potassium iodide. The solution is then titrated with standard silver nitrate. When a permanent faint turbidity has been obtained, both burets are read. One more centimeter of the standard cyanide is added, and again the end point is reached with standard silver nitrate. In the same manner as in the determination of the acid-base ratio, five end points are determined, and the data are used for the calculation of the cyanide-silver nitrate ratio. The numbers of the burets and the rates of delivery are incorporated into the record.

**TITERS OF STANDARD CYANIDE AND STANDARD SILVER NITRATE.** The volumes of standard silver nitrate used in the titration of silver for the determination of the titer of the cyanide solution are multiplied by the cyanide-silver nitrate ratio and then subtracted from the volumes of standard cyanide used. This gives the net volumes of standard cyanide required for reaction with the metallic silver taken. The ratios mg silver per CM standard cyanide are calculated. Their mean is taken as the titer of the standard cyanide, and the average deviation of this mean is the precision of the titer. The titer of the standard silver nitrate is obtained by multiplying the titer of the standard cyanide by the cyanide-silver nitrate ratio.

## EXPERIMENT 76

Determination of Silver and Copper in Coin Silver<sup>35</sup>

Coin silver, 90.0 per cent silver and 10.0 per cent copper, may be obtained in the form of fine wire. The weight of 1 cm of this wire is determined and listed on the label.

Precise balance, or pipet and solution containing known amounts of silver and copper.

Efficient centrifuge.

Siphon operated by suction, Fig. 15.

Apparatus and reagents employed in Expts. 74 and 75.

The experiment is an example of the use of microtitration in the estimation of the quantities of ions isolated in the course of qualitative analyses.<sup>34</sup> After the alloy has been dissolved in nitric acid, the silver ion is precipitated by adding ammonium chloride. The solution is removed with a capillary siphon, and the precipitate of silver chloride is washed. The precipitate is dissolved in ammonia, and the solution is titrated with standard cyanide. The copper is determined iodometrically in the centrifugate and washings.

The surface of the wire of coin silver is carefully cleaned, and then a length of it which corresponds to approximately 5-mg weight is cut off with clean shears. The wire is weighed on a balance for which the precision of a single weighing is  $\pm 0.025$  mg or better. The wire is transferred into a titrating cone. A bulb is placed on the opening of the cone, and the wire is dissolved in 0.2 ml of 3 *M* nitric acid. When solution is complete, the bulb is rinsed into the cone with 3 drops of water. The cone is placed on the steam bath, and the solution is treated with 5-λ portions of 1 *M* ammonium chloride until no more precipitate separates. Care should be taken to avoid an excess of the reagent larger than 5 λ. The flocculation of the precipitate is hastened by vigorous stirring while heating on the steam bath. The precipitate is collected in the point of the cone by means of an electric centrifuge, and the clear supernatant solution is siphoned off into another titrating cone. A siphon, Fig. 15, is used. The precipitate is washed with five 0.05-ml portions of hot 1.2 *M* nitric acid, and centrifugate and washings are collected for the determination of copper. If traces of silver chloride are carried over into the washings, they are collected by means of the centrifuge. The clear solution is transferred to another titrating cone. After washing, the silver chloride is dissolved in ammonia, and the solution is added to the main portion of the silver chloride precipitate.

For titration, the silver chloride is dissolved in 0.05 ml of 6 *M* ammonia. The solution is treated with 0.1 ml of water, 0.01 ml of

1 *M* potassium iodide, and titrated with the standard cyanide solution as outlined in the preceding experiment.

The solution containing the copper is treated with 1 drop of concentrated sulfuric acid and then evaporated to fumes of sulfur trioxide. The residue is dissolved in 0.3 ml of water. The solution is first made alkaline by adding ammonia, and then it is acidified with acetic acid and titrated as outlined in Expt. 74.

Results within the limits 89.8 to 90.2 per cent silver and 9.8 to 10.2 per cent copper may be considered satisfactory.



## APPENDIX

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## BASIC COURSE IN MICROTECHNIQUE

The following list of experiments may be considered a suggestion for a basic course in microtechnique which may be given without the use of microscopes. A few precise analytical balances should be available.

Preparation of apparatus, Expts. 1, 2, 3, 5, 7, 8, 9, 10.

Spot tests, Expts. 11 to 16.

Bead test, Expt. 34.

Determination of sensitivity, Expt. 35.

Work on the microscope slide, Expts. 37 to 39.

Work in the capillary, Expts. 43, 44, 46, 47.

Work in the microcone, Expts. 48 to 51.

Gravimetric experiments, Expts. 59 to 62, 64, 65, (70).

Titrimetric experiments, Expts. 71 to 74.

If one microscope is available for three workers, Expts. 4, 6, 20 to 28, 32, 33, and 36 should be added. If a microchemical balance is available, one gravimetric determination (Expt. 67) with the use of this balance should be added. The addition of Expts. 56 and 57 appears as the next most desirable.

## REAGENTS FOR QUALITATIVE ANALYSIS

### Stock Solutions

The stock solutions contain 50 mg of the test substance in 1 ml of solution. They are used for the preparation of the test solutions, the known solutions, and the unknown solutions. In the following table are listed the weights of solute which are required to make up 1 liter of stock solution. The proper solvents are given in the third column. These solvents must be also used when diluting the stock solutions in the preparation of test solutions.

Test substance	Solute to make 1 liter 50 mg/ml solution	Solvent
Ammonium ion	155 grams NH <sub>4</sub> Cl	Water
Antimony (III)	95 grams SbCl <sub>3</sub>	3 M HCl
Arsenic (V)	75 grams As <sub>2</sub> O <sub>5</sub>	Water
Bismuth	115 grams Bi(NO <sub>3</sub> ) <sub>3</sub> ·5H <sub>2</sub> O	3 M HNO <sub>3</sub>
Cadmium	135 grams Cd(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	Water
Cobalt	250 grams Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Water
Copper	190 grams Cu(NO <sub>3</sub> ) <sub>2</sub> ·3H <sub>2</sub> O	Water
Iron (III)	145 grams FeCl <sub>3</sub>	1 M HCl
Lead	80 grams Pb(NO <sub>3</sub> ) <sub>2</sub>	0.3 M HNO <sub>3</sub>
Mercury (I)*	70 grams Hg <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub> ·2H <sub>2</sub> O	1 M HNO <sub>3</sub>
Mercury (II)	117 grams Hg(NO <sub>3</sub> ) <sub>2</sub> ·8H <sub>2</sub> O	1 M HNO <sub>3</sub>
Phosphate, PO <sub>4</sub>	72 grams KH <sub>2</sub> PO <sub>4</sub>	Water
Silver	80 grams AgNO <sub>3</sub>	Water
Tin (IV)	140 grams SnCl <sub>4</sub> ·5H <sub>2</sub> O	6 M HCl

\* This solution will not keep longer than 1 year.

### Test Solutions

The test solutions contain 10 mg of the test substance in 1 ml of the solution. They are prepared by diluting the above listed stock solutions to five times their volume with the solvent listed in the table on stock solutions. In addition the following are prepared:

*Borate test solution*, by dissolving 16 grams of borax, Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O, in water to make 1 liter of solution.

*Bismuth-lead test solution*, by dissolving 1.2 gram Bi(NO<sub>3</sub>)<sub>3</sub>·5H<sub>2</sub>O and 8 grams Pb(NO<sub>3</sub>)<sub>2</sub> in 3 M nitric acid to make 100 ml of solution.

The test solutions are filtered off into 1-oz bottles which are provided with a special kind of label to make them readily recognizable.

## Known Solutions

The known solutions are prepared by mixing the volumes of stock solutions given in column 4 of the following table, and finally diluting the mixture to a volume of 100 ml with the diluent listed in column 5. The solutions are kept in 1-oz bottles provided with the type of label used for test solutions.

Known solution	Substance	Concentration in known solution grams per liter	Stock solution ml	Diluent
I *	Silver	10	20	1 M HNO <sub>3</sub>
	Mercury (I)	10	20	
	Lead	10	20	
II	Mercury (II)	2	4	3 M HNO <sub>3</sub>
	Lead	5	10	
III	Silver	2.5	5	Water
	Mercury (II)	5	10	
	Lead	10	20	
	Conc. HNO <sub>3</sub>		20	
	Bismuth	5	10	
	Copper	2.5	5	
	Cadmium	10	20	
IV	Mercury (II)	2.5	5	Water
	Copper	2.5	5	
	Conc. HCl		25	
	Arsenic	2	4	
	Antimony	5	10	
	Tin	5	10	
V	Bismuth	1	2	Water
	Antimony	10	20	
	Conc. HCl		25	
VI	Arsenic	2.5	5	Water
	Antimony	5	10	
	Conc. HCl		50	

\* Known solution I seems to be supersaturated with respect to some double salt. If crystallization occurs, the solution is rejected.

## Unknowns

Unknown solutions are made up in the same manner as the known solutions. They are put into 1-oz bottles, and the labels with the key numbers

are best attached to the bottoms of the bottles. The solutions should be in the charge of the instructor. When a student needs an unknown solution, he brings a clean, dry calibrated capillary pipet or centrifugal pipet to the instructor, who measures off the required volume of solution.

For the purpose of self-instruction, the unknowns are best prepared at the outset. Of each type, two or three different solutions of identical appearance are prepared, and the labels with the key numbers are attached to the bottoms of the bottles. The composition of the unknowns is recorded on cards, a separate card being used for each unknown. Each card is sealed in a separate envelope which shows the key number on the outside. When a sample is taken for analysis, the bottle is provided with an identification mark without looking at the key number.

*Unknown silver solution* (Expt. 48) should contain from 0.5 to 10 mg of silver per milliliter solution.

*Unknown on copper group* (Expt. 51) should not contain more than two or three of the elements mercury (II), silver, lead, bismuth, copper, and cadmium.

*Unknown on copper and arsenic groups* (Expt. 53) should contain one or two elements of each subgroup.

*Alloys for unknowns* (Expt. 55). The following alloys containing metals of the copper and arsenic groups are recommended: coin silver (Ag, Cu), solder (Sn, Pb), bronze (Cu, Sn, Pb), Rose's alloy (Bi, Pb, Sn), Babbitt metal (Sn, Pb, Sb, Cu), and Wood's alloy (Bi, Cd, Pb, Sn).

The alloys may be supplied in the form of filings. Silver-copper alloys may be obtained from Baker and Co., 54 Austin Street, Newark, N. J., in the form of very fine wire. Wood's alloy and Rose's alloy may easily be made into the form of fine wire by pouring them into glass capillaries which are broken after the alloy has solidified. Of course, the quantitative composition of the alloys should be known with an accuracy corresponding to two significant figures.

#### General Reagents

The reagents listed below are generally needed. Most of them may be supplied in 1-oz bottles; exceptions are indicated. It is obvious that it would be sufficient to supply concentrated reagent solutions only. It will be found, however, that the continuous need for the preparation of various dilutions is annoying and leads to a considerable waste of time and material. To save clerical work, the students may be supplied with the clean, labeled bottles, and whenever the need arises, the students prepare the required dilution in larger quantity and put it into the bottles which are held ready for this purpose. All the reagents should be supplied chemically pure. Since very little of the reagents is needed, the expense is negligible. Solutions in which a sediment has formed should be immediately rejected and replaced.

If not directed otherwise, solid reagents should be supplied in 2-gram quantities in screw-cap vials. Granules 0.1 to 1 mm in diameter are preferred. They are obtained by crushing larger crystals in a mortar and classifying the material

on a sheet of paper so that the coarser grains and the fine powder are rejected. This applies also to all solid reagents employed for slide tests (see below).

Acetic acid, glacial, and 2 *M*.

Chromic-sulfuric acid, cleaning solution, large volume.

Hydrobromic acid, 9 *M*.

Hydrochloric acid, 12 *M*, 6 *M*, 3 *M*, 2 *M*, and 12 *M*.

Nitric acid, 16 *M*, 6 *M*, 3 *M*, 2 *M*, and 12 *M*.

Phosphorous acid, 3 *M*.

Sulfuric acid, 18 *M*, 6 *M*, 3 *M*, 0.6 *M*.

Hydrogen sulfide, pure, supplied with a pressure of 1.03 atm (30 cm water column in excess of atmospheric pressure).

Sodium hydroxide, solid in pellet form, 6 *M*, 3 *M*, 2 *M*, and 0.6 *M*. The solutions must be frequently renewed.

Ammonia, 14 *M* (concentrated), 6 *M*. The solutions should be frequently renewed. Concentrated ammonia for "fuming" should be supplied in special 250-ml bottles.

Acetone, free from residue.

Ammonium acetate, 6 *M*, 3 *M*.

Ammonium chloride, 3 *M*, 1 *M*.

Ammonium chromate, 2 per cent solution, 20 ml per student.

Ammonium oxalate, saturated solution. At 15° C 1 liter of water will dissolve approximately 40 grams of  $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$ .

Ammonium sulfide, 7 *M*. Concentrated ammonia is saturated with hydrogen sulfide and then treated with an equal volume of the ammonia.

Ammonium thiocyanate, saturated solution.

Amyl alcohol, iso, b.p. 130° C.

Anhydrene, 100 grams.

Ascarite, 100 grams. In filling adsorption tubes, the chemical agent is always placed between two wads of cotton or glass wool which retain dust particles and keep the filling in place. The end of the Ascarite charge, which faces the inlet tube for the gas, is treated with a drop of water.

Beeswax, 100 grams.

Canada balsam, 1 tube.

Carbon tetrachloride, 100 ml.

Cedarwood oil, 1 oz.

Copper wire, 0.1-mm diameter, B. & S. 42, pure electrolytic copper. Approximately 1 gram of it (50 feet) is kept in a jar with screw cap. The weight of a 1-cm length of the wire is listed on the label.

Ethyl alcohol, 95 per cent, no residue, 100 ml.

Glycerin. Each student should have within reach a 2-oz bottle one-third full of glycerin. The stopper is provided with a 4-mm glass rod which nearly reaches to the bottom of the bottle.

Magnesium ribbon, 10 cm per student.

Methyl red, *p*-dimethylaminoazobenzene-*o*-carboxylic acid; 0.1 *M* sodium hydroxide is saturated at room temperature with the solid dye.

Potassium bromate, solid.

Potassium cyanide, 10 per cent solution.

Potassium dichromate, 0.1 *M* (3 per cent), 0.02 *M*.

Potassium iodide, solid.

Potassium permanganate, crystals, 50 grams.

Soap solution for cleaning purposes, 1 liter.

Sodium sulfide-hydroxide reagent: 480 grams  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  and 40 grams NaOH are dissolved in water to make 1 liter of solution.

Stannous chloride: 115 grams  $\text{SnCl}_2\cdot 2\text{H}_2\text{O}$  are dissolved in 170 ml of concentrated HCl. The solution is diluted with water to make 1 liter. Some metallic tin is placed in every bottle containing this reagent.

Stannite reagent is always prepared freshly before use: 3 to 4 drops of stannous chloride are added to approximately 6 ml of 2 *M* sodium hydroxide (Expt. 26).

### Special Reagents

The following reagents are needed only for certain experiments and may be omitted if performance of these experiments is not contemplated.

EXPT. 32. Turmeric linen. Five grams of powdered turmeric is refluxed for 5 minutes with 10 grams of 95 per cent ethyl alcohol. The solution is filtered and evaporated. The residue is dissolved in 4 ml 50 per cent alcohol, and the solution is treated with small portions of solid sodium carbonate until it becomes clear. Unbleached linen fibers are placed in this solution, which is then heated just to boiling. The fibers are removed from the bath and dried by pressing between filter paper. The fibers are placed for 30 seconds in 1 *M* sulfuric acid and then carefully washed with water, pressed between filter paper, and spread for drying between two sheets of filter paper. The fibers should have the color of egg yolk.

EXPT. 33. Sodium sulfide, 1 per cent solution of  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ .

EXPT. 34. Borax, powder.

EXPT. 36. Carbon-aluminum oxide mixture. One part (by weight) of bone black is mixed with 99 parts of aluminum oxide. The particle size of the constituents is determined under the low-power microscope before preparing the mixture. If either black or white particles are too small, it becomes impossible to separate them by following the instructions given in the experiment.

EXPT. 40. Chloroplatinic acid solution containing 5 per cent of  $\text{H}_2\text{PtCl}_6\cdot 6\text{H}_2\text{O}$ . The reagent should be kept in a small bottle of clear fused quartz. From time to time the reagent should be tested by placing 5  $\lambda$  of it on a microscope slide and allowing it to evaporate at room temperature. The residue is examined under the microscope for crystals of potassium or ammonium chloroplatinate. It is needless to point out that ammonia must be absent from the air in which the test is carried out.

EXPT. 42. Potassium iodide, 0.1 *M*.

EXPT. 43. Iodine, fine powder.

EXPT. 54. Ether saturated with 6 *M* hydrochloric acid. An 8-oz bottle is provided with graduation marks corresponding to 120 and 200 ml. First 6 *M* hydrochloric acid is poured into the bottle until the 120-ml mark is reached, and then ether is added up to the 200-ml mark. The bottle is closed and the contents are mixed by shaking. The ether may be replenished several times before it becomes necessary to renew the acid.

Ammonium (or potassium) thiocyanate, 1 *M*.

## ADVANCED WORK:

Hydrofluoric acid, no residue.

Potassium pyrosulfate.

Sodium carbonate, anhydrous.

Sodium peroxide.

## Reagents for Spot Tests

**Ammonium phosphomolybdate paper.** Strips of filter paper (Schleicher & Schüll, No. 589<sup>8</sup>) are soaked in a 1 per cent aqueous solution of phosphomolybdate acid. A few milliliters of concentrated ammonia are poured into a 400-ml beaker, and each strip of paper is exposed for 10 to 20 seconds to the ammonia fumes by holding it in the gas space of the beaker immediately after removing it from the phosphomolybdate acid solution. The strips are allowed to dry, and they are then collected and placed in bottles of amber glass.

**Cadion 3B**, benzenediazoaminobenzene-4-azo-4'-nitrobenzene, 0.02 per cent solution in 95 per cent ethyl alcohol.

**Cinchonine-iodide reagent.** (a) One gram cinchonine hydrochloride is dissolved in 19 ml water, and 1 ml 6 *M* hydrochloric acid is added; (b) 2 grams potassium iodide is dissolved in 10 ml water. Before use 2 volumes of (a) are mixed with 1 volume of (b).

**Iodide-acetate reagent.** Twenty-five grams of ammonium acetate and 10 grams of potassium iodide are dissolved in 50 ml of water.

**Potassium cyanide**, solid, granules.

**Potassium hydroxide**, alcoholic solution. Eighty milliliters of 2 *M* potassium hydroxide are mixed with 20 ml 95 per cent alcohol.

**Potassium iodide paper.** Drop test paper is soaked in 1 per cent potassium iodide solution and then allowed to dry. The dry reagent paper is cut into strips and placed in amber bottles.

**Quinine-iodide reagent.** (a) One gram quinine hydrochloride is dissolved in 50 ml warm water. The solution is treated with 0.2 ml 6 *M* hydrochloric acid. (b) Two grams of potassium iodide is dissolved in 50 ml of water. Before use, equal volumes of (a) and (b) are mixed.

**Rochelle buffer solution.** Ten grams of Rochelle salt is dissolved in water to give 100 ml of solution, which is then treated with 0.1 ml of glacial acetic acid.

**Rubeanic acid**, dithiooxalicdiamide, 1 per cent solution in 95 per cent ethyl alcohol.

**Silver nitrate-acetate buffer solution.** One gram of silver nitrate and 7.7 grams of ammonium acetate are dissolved in a mixture of 6 ml of glacial acetic acid and 200 ml of water.

**Sodium chloride**, 1 per cent aqueous solution.

## Reagents for Slide Tests

**Acetate buffer solution.** One treats 450 grams of sodium acetate trihydrate with 100 ml glacial acetic acid and adds water to make 1 liter of solution.

**Ammonium-cobaltous thiocyanate reagent.** Ten grams of cobaltous nitrate hexahydrate and 10 grams of potassium thiocyanate (or 8 grams of ammonium thiocyanate) are dissolved in 33 ml of water.

Cesium chloride, solid, granules.

Magnesium acetate, solid.

Potassium cobalticyanide, solid, granules. The preparation of this salt is described in *Z. anal. Chem.*, 70, 258 (1927).

Potassium dichromate, solid, granules.

Potassium ferrocyanide trihydrate, solid, granules.

Potassium nitrite, approximately 6 M solution. One dissolves 500 grams of potassium nitrite in water to give 1 liter of solution.

Reinecke's salt, potassium tetrathiocyanodiamminochromate, crystals.

Rubidium chloride, solid, granules.

Silver chloride, solid, in amber vial.

Thiourea, 5 per cent aqueous solution.

## REAGENTS FOR GRAVIMETRIC EXPERIMENTS

### Test Substances

Aluminum alum,  $KAl(SO_4)_2 \cdot 12H_2O$  or  $NH_4Al(SO_4)_2 \cdot 12H_2O$ , tested purity, crystals of less than 1-mm diameter. Alum has little tendency to lose water. Microscopic inspection will show whether dehydration has taken place. The double salt may be recrystallized from hot water as outlined for magnesium sulfate.

German silver, fine filings, analyzed. Certified samples for microanalysis should be prepared from selected small pieces of the alloy so as to be reasonably certain about the absence of segregation. The filings should be carefully mixed and freed from iron. If suitable samples cannot be obtained, a test solution may be prepared as follows. Exactly 0.600 gram electrolytic copper is transferred to an Erlenmeyer flask, the opening of which is covered with a glass bulb. The metal is treated with 20 ml water, 8 ml 16 *M* nitric acid, and 4 ml 18 *M* sulfuric acid. When the copper has completely dissolved, the bulb is rinsed into the flask. Exactly 1.077 gram of  $(NH_4)_2Ni(SO_4)_2 \cdot 6H_2O$  and 1.056 gram of  $ZnSO_4 \cdot 7H_2O$  are added to the solution which is diluted with 40 ml of water and then boiled for 1 minute. After the addition of 0.5 gram of urea the boiling is continued for 2 more minutes. The solution is cooled to room temperature, quantitatively transferred to a volumetric flask, and diluted with water to make exactly 100.00 ml. One milliliter of this solution corresponds to 10.00 mg of German silver consisting of 60.00 per cent copper, 16.00 per cent nickel, and 24.00 per cent zinc. The volume of solution required for analysis is determined by the precision of the balance used. The solution is simply transferred to the electrolytic beaker and diluted with water until the electrodes are properly immersed.

Iceland spar, primary standard, ground to fine powder.

Limestone, fine powder, analyzed. Certified samples for microanalysis should be prepared from reasonably homogeneous-looking pieces of limestone. The material is finely ground, and the resulting powder is carefully mixed. The limestone should contain 30 to 40 per cent calcium oxide, 2 to 20 per cent magnesium oxide, and 2 to 5 per cent silica.

Magnesium sulfate heptahydrate, tested purity, small crystals. Tendency to dehydration is very pronounced, and the crystals must be inspected under the microscope before use. White spots indicate partial dehydration. The safest procedure is recrystallization of a small quantity before use. Approximately 1 gram of the salt is placed in a microbeaker and dissolved in boiling water. The beaker is then set aside and allowed to cool. Needles a few millimeters long separate. A small funnel with a short stem a few tenths of a millimeter in bore is drawn out from glass tubing of 8- to 10-mm bore. The crystals are transferred with the mother liquor into the funnel, and the solution is allowed to drain off. The funnel is then placed on the opening of a centrifuge cone, and the two are whirled in an efficient centrifuge. The

crystals are obtained in a perfectly dry state, and they are transferred to a screw-cap vial by inclining the funnel and tapping its outside. The advantages of centrifugal drainage in ridding crystals of mother liquor have been pointed out by T. W. Richards, *J. Am. Chem. Soc.*, **27**, 104 (1905). Techniques adapted to the treatment of small quantities of crystals are described in the books of Emich and Pregl and in articles by Arnulf Soltys, *Mikrochemie, Molisch-Festschrift*, p. 393 (1936), D. F. Houston and Ch. P. Saylor, *Ind. Eng. Chem., Anal. Ed.*, **8**, 302 (1936), Th. Perrine and Wm. Kump, *ibid.*, **11**, 658 (1939), C. R. Johnson and Harvey Miller, *ibid.*, **13**, 118 (1941), and Lyman C. Craig, *ibid.*, **12**, 773 (1940).

Selenite,  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ , light-colored clear plates of the mineral.

Sodium oxalate, Sørensen, primary standard.

### Reagents

**Acids.** The customary specifications on purity of reagents used in quantitative work are satisfactory in general. It is preferable, however, to redistill the small volumes of acid required. Approximately 25 ml of concentrated nitric acid or sulfuric acid is placed in a 100-ml distilling flask of clear fused quartz or Pyrex glass, which has the side arm set on the center of the neck. A microbeaker of quartz or Pyrex, which is half full of cold water, is placed in the neck so that it rests with the rim on the opening of the neck. No condenser is required, and the acids are collected in 50-ml bottles of clear fused quartz which are provided with an inverted stopper of the cap type.

Concentrated hydrochloric acid is prepared by passing hydrogen chloride into freshly distilled water. The gas is obtained by heating concentrated acid. Approximately 150 ml of concentrated hydrochloric acid is placed in a 300-ml round-bottom flask and treated with 2 ml of chromous chloride solution (hydrochloric acid solution of chromic chloride reduced with metallic zinc). A porous tile or a capillary is added to prevent bumping, and the flask is closed by a rubber stopper with a  $\Lambda$ -shaped tube of clear fused quartz or Pyrex glass. The free end of the long leg of the  $\Lambda$ -tube is inserted in 50 ml of redistilled water held ready in a quartz bottle which is best placed in a funnel so that it becomes possible to run cold water over the outside for the purpose of cooling. The acid in the round-bottom flask is heated by fanning the flask with a Bunsen flame. The bottle is lowered and the heating is stopped as soon as a noticeable amount of reflux in the  $\Lambda$ -tube indicates that constant-boiling acid has begun to distil over. In the place of the makeshift apparatus, use of a Pyrex still with standard ground joints is recommended. It may consist of flask No. 4320, connecting tube No. 8980, and an 8-mm tube in place of a condenser. Various apparatus for the quick preparation of small quantities of pure water and liquid reagents have been described by F. Emich, *Methoden der Mikrochemie*, in *Abderhalden's Handbuch der biologischen Arbeitsmethoden*, Abt. I, Teil 3, pp. 45-324, Vienna and Berlin, Urban u. Schwarzenberg, 1921.

Acid hydrofluoric, conforming to the specifications of the American Chemical Society.

Ammonia attacks glass very strongly, and consequently the reagent becomes contaminated. A pure ammonia solution is easily prepared, however, by distillation as outlined for the preparation of pure hydrochloric acid. Ap-

paratus of Corning Brand Alkali Resistant Glass No. 728 is recommended. The reagent should be stored in a bottle of this glass or in a paraffin bottle with a glass cap instead of the stopper.

Ammonium acetate, 2 *M*. The solution must be perfectly clear. If necessary, small portions of it are centrifuged before use.

Ammonium chloride, 0.6 *M*. Thirty grams of ammonium chloride is dissolved in water to give 1 liter of solution. The solution must be clear.

Ammonium citrate, solid, 25 grams.

Asbestos, purified for use with Gooch crucibles, soft long fibers.

Calcium chloride hexahydrate, 500 grams, for use in drying tower.

Carbon dioxide snow, dry ice. Prepared or ordered before use.

Inert gas, supply of either hydrogen, nitrogen, or carbon dioxide.

Methyl alcohol, 100 ml, free from residue.

Oxalic acid, clear 3 per cent solution of  $C_2H_2O_4 \cdot 2H_2O$ .

Oxin reagent. Eight grams of 8-hydroxyquinoline (analytical reagent quality) are triturated in a mortar with 12 grams of glacial acetic acid. The mixture is dissolved in 83 ml of water. The solution is filtered or centrifuged to make it perfectly clear. It is kept in an amber bottle. After 3 months there may be signs of decomposition. If so, a new batch of reagent is prepared.

Sodium carbonate, anhydrous, tested purity.

Sodium phosphate, secondary,  $Na_2HPO_4 \cdot 12H_2O$ . One hundred grams of the salt is dissolved in water to give 1 liter of solution. The solution is filtered or centrifuged.

Sodium-potassium carbonate, anhydrous, tested purity. A 1 : 1 (by weight) mixture of the two salts.

## REAGENTS FOR TITRIMETRIC ANALYSIS

### Standards and Test Substances

Copper wire, *see* Qualitative reagents.

Silver wire, 0.1-mm diameter, B. & S. 42, of pure silver, of coin silver, and other silver copper alloys of definite composition may be obtained from Baker and Co., 54 Austin Street, Newark, N. J. The wires are kept in jars with screw caps. Composition and weight of 1-cm lengths of the wires should be given on the labels.

### Standard Solutions

Acid. Approximately 0.5 *M* hydrochloric acid.

Base. Approximately 0.5 *M* sodium hydroxide obtained by diluting oily lye (100 grams of NaOH is dissolved in 100 ml distilled water. After standing for 14 days, the clear liquid is siphoned off and stored in a stoppered bottle).

Potassium cyanide, approximately 0.2 *M*. Thirteen grams KCN and 5 grams KOH are dissolved in water to give 1 liter of solution. The titer has to be determined every other day.

Silver nitrate, approximately 0.1 *M*.

Sodium thiosulfate, approximately 0.1 *M*. Twenty-five grams of the pentahydrate is dissolved in 1 liter of water, and 0.4 ml of chloroform is added.

### Reagents

Mercury, pure and dry. One milliliter of it suffices.

Methyl red, *see* Qualitative reagents.

Potassium iodide, 1 *M*.

Potato starch. The indicator solution is prepared by triturating 0.5 gram of the starch with 5 ml of water and pouring the thin paste in 25 ml of boiling water. Boiling is continued until a clear solution is obtained. One gram of potassium iodide is added, and the solution is quickly cooled to room temperature. Mold will appear within a few days.

## LIST OF APPARATUS

### Basic Supply of the Individual Workbench

Air supplied under low pressure. Water blower, Fig. 13.

Beakers, Pyrex: 1 low form, Griffin, 400-ml; 3 low form, Griffin, 150-ml; 1 Phillips, 250-ml.

2 bottles with glass stoppers, 1-oz, one for distilled water and one for concentrated hydrochloric acid. These reagents are used for the cleaning of platinum loops.

1 box, cardboard, 35 cm long, for storing capillaries.

1 Bunsen burner.

1 Bunsen burner with pilot flame.

1 buret clamp.

3 camel's-hair brushes, small.

Centrifuge tubes: either 12 microcones, Fig. 4B, and 6 straight-tip cones, Fig. 4C, 4 with 1-mm-bore measuring capillary and 2 with 2-mm-bore measuring capillary, or 12 centrifuge cones, Fig. 4A, and drain rack.

2 connecting tubes, tapered, Fig. 14Ac.

Cotton in jar.

File and a few pieces of broken porcelain or glazed earthenware.

1 filtering flask, heavy wall, with side tube, 250-ml.

Filter paper, a few disks of any kind of filter paper. If spot tests are to be performed, the following are added: Schleicher & Schüll, No. 589<sup>3</sup> (blue ribbon), and Schleicher & Schüll, drop test paper No. 601.

Flasks, Erlenmeyer, one 125-ml and one 250-ml capacity.

Forceps, preferably made from stainless steel and having smooth parallel tips not corrugated or toothed. Recommended: La Cross, No. 1013-3½, flat tips, manufactured by Schnefel Bros., Newark, N. J.

Glass ring, 1 cm high and 2 cm in diameter, ends cut square and ground, Fig. 31.

Graduated cylinder, 10-ml.

Hardwood universal block, Fig. 6.

Litmus paper.

Magnifying glass.

Measuring pipets: one 2-ml divided into 0.1 ml, and two 0.2-ml divided into 0.01 ml.

Millimeter ruler, celluloid, 20 cm long.

Paper, glazed, 5 cm square, white, black, green, and red.

Platinum wire: 3 cm of B. & S. 31, 0.3-mm diameter, and 3 cm of B. & S. 47, 0.05-mm diameter.

2 probing wires, 0.8- to 1-mm steel wire with knurled ends.

1 ring stand.

Rubber stoppers: one No. 4, 2 holes; four No. 5, 1 hole; one No. 6, 2 holes.

Scissors.

2 screw clamps.

Slides: 12 microscope slides, 25 mm by 75 mm, of clear non-corrosive glass.

3 narrow slides, 8 mm by 75 mm, cut from microscope slides.

2 slides 5 cm square, of thick glass.

1 ground-glass slide. A poor but satisfactory substitute is easily obtained by sandpapering a microscope slide.

Soap, 1 cake.

1 or 2 spatulas. The Double End Macro and Semimicro Spatula No. 19744 of the Central Scientific Company is made of pure nickel. Smaller spatulas are obtained by flattening the end of copper, nickel, or platinum wire of 0.5-mm diameter, B. & S. 25.

Stand for suction tube, Fig. 15D.

Steam-bath device, Fig. 7.

Suction control tube, Fig. 15B.

Suction pump and trap, Fig. 12.

Test tubes: 12 Pyrex test tubes, 6 inch; 1 test tube with side arm, Fig. 15A.

Test-tube brush.

Test-tube rack.

Thermometer, short, 350° divided in 5° C.

2 towels.

Triangle, clay or quartz.

Tripod.

Watch glasses: two 1-in. diameter, and two 3-in. diameter.

Wire gauze.

#### General Supply

Agate mortar and pestle.

Analytical balance and set of weights.

Beeswax or sculptor's wax (Plasticine) for a substitute.

Blast lamp.

Bottles for reagents. Pyrex bottles No. 1560 with inverted (cap-type) stoppers are recommended.

Buzzer, p. 19.

Capillary clamps, Fig. 38. For substitutes see Expts. 8 and 46.

Centrifuges. Recommended: Clay-Adams Company No. CT-1100/D Adams Senior Standard Centrifuge equipped with two CT-2180 aluminum centrifuge shells with open bottoms for micro work in place of 15-ml shells. Also two No. CT-2150 aluminum shells for 2-, 1-, and 0.5-ml test tubes. See p. 13.

Cork borer, set.

Distilling devices: centigram scale, device Fig. 39; milligram scale, device Fig. 44.

Feathers, supply, for cleaning centrifuge cones.

Forceps with locking device.

Gas pipes, 15 cm long, closed at one end, Expt. 55.

Glass rod, supply, 4-mm and 6-mm diameter.

Glass tubing, supply: soft-glass tubing of 6-mm, 8-mm, 10-mm, and 15-mm outer diameter. See Expt. 2 for the testing of glass on its suitability for the preparation of capillaries.

Pyrex tubing of 24-mm outer diameter.

Goggles.

Heating blocks, aluminum, Fig. 9.

Millimeter graph paper, supply.

Paper towels, supply.

Paraffin blocks.

Platinum foil. A small supply of platinum foil is desirable. Foil 0.05 mm (0.002 in.) thick and 0.008 mm (0.0003 in.) thick, free from pores, is recommended.

Pressure caps, Fig. 10.

Rubber tubing. Large supply of flexible tubing of 3-mm bore and 2-mm wall.

Short pieces (15 mm long) of soft tubing of 1-mm bore are needed. Rubber squares of 1-cm edge are cut from rubber tubing used for Gooch crucibles.

Sandpaper, supply.

Thermometer capillary of uniform round bore, Pyrex, 1-mm and 2-mm bore.

Toothpicks, supply.

Vials with screw caps of Bakelite, 8-ml capacity, are recommended as containers for solid reagents.

Vials, straight wall, 18-mm diameter, small supply.

Vise, small, and steel needle of 1-mm diameter.

Wires:

Aluminum, B. & S. 19 (1 mm) and B. & S. 25 (0.5 mm).

Copper, B. & S. 19 (1 mm).

Nichrome, B. & S. 25 (0.5 mm).

Nickel, B. & S. 25 (0.5 mm).

Platinum, B. & S. 25 (0.5 mm).

### Apparatus for Microscopy

Microscopes. For slide tests and general microchemical work, a microscope of the following specifications will suffice for two to three students: simple but rugged stand of standard size and shape; coarse adjustment but not necessarily a fine adjustment; 2 objectives having magnifications 4 and 10, respectively; micrometer eyepiece of the approximate magnification 8; combination of plane and concave mirror which can be rotated and swung to the left and right; no condenser and no diaphragm, but a ring underneath the stage, which permits attachment of a standard condenser or polarizer; no tilting mechanism required.

A very reasonably priced microscope of these specifications is manufactured by C. Reichert (Pfaltz & Bauer, Inc., Empire State Building, New York). A simple rotating stage can be attached. Addition of Nicol prisms or Polaroid plates quickly converts any instrument of this type into a reasonably satisfactory polarizing microscope.

Microscope lamps, see p. 27.

Cells for microcones, Fig. 43.

Cover slips.

Lens paper.

Stage micrometer, or substitute described in Expt. 6.

### Apparatus for Micromanipulation

The following suggestions supplement the specifications given on pages 141 to 151.

Microscopes. The Large Compound Mechanical Stage E of Carl Zeiss, Inc., 485 Fifth Avenue, New York, N. Y., and one of the Zeiss stands F, FZ, G, or S, all of which permit use of the above-mentioned mechanical stage, are recommended. Equally satisfactory is the equipment furnished by E. Leitz, Inc., 730 Fifth Avenue, New York, N. Y.: Rotary Mechanical Stage "Peeyg" No. 22611 and microscope AM-R with monocular body tube or microscope AMBI-R with inclined binocular body tube. The use of cup grease is recommended, if it should become necessary to lubricate the mechanical stage.

Manipulators. The Simplified Manipulator "Mifyxir" of E. Leitz was used in all our work. Inquiries concerning suitable substitutes may be directed to J. H. Emerson, 22 Cottage Park Avenue, Cambridge, Massachusetts; to Rayno Research, 3806 North Ashland Avenue, Chicago, Ill.; and to Oscar F. Soetheer, Massapequa Park, L. I., N. Y.

Pipet holder and copper tubing may be ordered from E. Leitz. They are part of the Micro Injection Apparatus "Mandi."

Du Bois Automatic Device "Mifywor" for Drawing Needles and Pipets with regulating transformer "Redyx" for 110 to 220 volts alternating current may be ordered from E. Leitz.

Inquiries concerning the plunger device and Rachele's device for drawing micro-pipets may be directed to Mr. Julius Nielsen, Department of Physics, Queens College, Flushing, N. Y.

### Apparatus for Gravimetric Work

By means of the check lists given with the experiments, it is easily possible to assemble sets of apparatus and reagents for the various determinations. The number of these sets will more or less determine the number of students who can work simultaneously.

Aluminum wire for the preparation of tares, B. & S. 14 (2-mm diameter), and B. & S. 19 (1-mm diameter).

Auxiliary masses, 10-mg and 20-mg pieces, used in the calibration of weights.

Balances: analytical balances of high precision, or microchemical balances, *see* p. 174. Each balance should be equipped with a set of weights, two 60-mm Petri dishes lined with velvet as supports for tare pieces in the balance case, an aluminum frame shown in Fig. 70, and a large brush for cleaning.

Bell jar, glass, approximately 10-cm diameter, glass plate.

2 pieces of chamois, 12 cm square or larger, in jar with screw cap.

Crucibles: Platinum, 1-ml capacity, with lid and tare. If several are available, crucible, lid, and tare should carry numbers. The best tare is made of platinum or gold.

Platinum, 15-ml capacity, with lid. Bulb and steam bath, Fig. 75.

Porcelain, high form, approximately 10 ml.

Crucible furnace, electric, or electric muffle is desirable.

Drying block, aluminum, and drying tube, Fig. 74.

Drying oven or Stähler block.

Drying tower, glass, for use with calcium chloride hexahydrate.

Electric charges, means for their dispelling, *see* p. 193.

Electrolytic apparatus, Fig. 76, complete with electrodes.

Evaporating dish, quartz, 8-cm diameter, or large porcelain crucible as a substitute. Expt. 61.

Filter paper, quantitative, Schleicher & Schüll, No. 589<sup>1</sup> (black ribbon), Expt. 69.

Flannel, 10 cm by 20 cm, in jar with screw cap.

Forceps: Special forceps, Figs. 71 and 72.

Cork-tipped. The flat tips of La Cross forceps are easily provided with cork surfaces. Chips of cork, 1 mm thick, are sliced from a stopper with a razor blade. The tips of the forceps are slightly heated over a microburner, and some Krönig glass cement is applied by touching the stick of cement to the hot metal. The cement melts, and the thin slices of cork are immediately placed on the tips, which are squeezed together until the cement has solidified. The excess of cork is trimmed off with the razor (M. Cefola).

Platinum-tipped. One pair for each 1-ml crucible.

Fritted glass, irregular pieces of approximately 2-mm diameter are obtained from broken apparatus containing disks of fritted glass. Porous porcelain or Alundum may be used also. See Expt. 64.

Gas washing bottles

Gauze for cleaning the interior of the balance cases.

Glass cement, Kröning, 1 weight of white beeswax and 4 weights of rosin.

Heating bar, aluminum, Fig. 67.

Linen, old, for cleaning balances.

Microbeakers, 7-ml, Fiolax glass, Fig. 69. Glass tare, filterstick, and microbeaker should be numbered.

Microbeaker, 15 ml, clear fused quartz, with Pyrex bulb to fit.

Microdesiccator, Saschek, Fig. 66.

Quartz triangle.

Rubber gloves or finger cots.

Suction device, Fig. 154.

Test tubes, 6-in., 16-mm diameter, Fiolax (black stripe) from Schott & Gen., Jena, Germany. Supply for the preparation of microbeakers. See Expt. 64.

Vials, straight wall, 18-mm diameter, small supply.

Wash bottle, 125-ml, with trap and inverted stopper (cap type); Pyrex No. 1720 is desirable but not necessary.

Wash bottle, calibrated, 10-ml, is best made from a 10-ml graduated cylinder.

Weighing tube, Fig. 77.

Wire clamp for test tubes.

#### Apparatus for Titrimetric Work

Analytical balance of high precision. As a substitute one may make use of standard solutions for knowns, and one may measure them off with micro-pipets, p. 238.

Containers for standard solutions, 300-ml capacity, operating by the principle used with the wash bottle. Rubber bulbs (bellows) supply the required pressure. An Ascarite tube is to be used to protect standard base against the carbon dioxide of the atmosphere. Standard base may be kept in a paraffin bottle.

Elevating stand with rack-and-pinion motion of the vertical rod is desirable.

Elevating stand with delicate motion S.750 and extension rod S.753 of The Gaertner Scientific Corporation, Chicago, Ill., are suggested.

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